Gypsy Moth Management in the United States:
a cooperative approach

Final
Supplemental Environmental
Impact Statement

Volume IV of IV

Appendixes J-M
Risk Assessments and Risk Comparison

United States
Department of Agriculture

Forest Service

Animal and Plant Health
Inspection Service

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a cooperative approach

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Abstract: The USDA Forest Service and Animal and Plant Health Inspection Service are proposing an addition to the gypsy moth management program that was described in the 1995 Environmental Impact Statement—Gypsy Moth Management in the United States: a cooperative approach—and chosen in the 1996 Record of Decision. The agencies are proposing these new treatment options: adding the insecticide tebufenozide, or adding the insecticide tebufenozide and other new treatment(s) that may become available in the future to manage gypsy moths, provided that the other treatment(s) pose(s) no greater risk to human health and nontarget organisms than are disclosed in this Final Supplemental Environmental Impact Statement for the currently approved treatments and tebufenozide. The addition of tebufenozide or other new treatment(s) to the list of approved treatment options does not change any program or administrative requirements identified in the 1995 EIS. Those requirements include any consultations required and the need to conduct site-specific environmental analyses in accordance with the National Environmental Policy Act and agency regulations.

The complete Final Supplemental Environmental Impact Statement consists of four volumes:

Volume I	Summary
Volume II	Chapter 1. Purpose of and Need for Action
	Chapter 2. Alternatives Including the Preferred Alternative
	Chapter 3. Affected Environment
	Chapter 4. Environmental Consequences
	Chapter 5. Preparers and Contributors
	Chapter 6. Mailing List
	Chapter 7. Glossary
	Chapter 8. References
	Appendix A. Gypsy Moth Treatments and Application Technology
	Appendix B. Gypsy Moth Management Program
	Appendix C. Scoping and Public Involvement
	Appendix D. Plant List
	Appendix E. Biology, History, and Control Efforts for the Gypsy Moth
Volume III	Appendix F. *Bacillus thuringiensis kurstaki* (*B.t.k.*) Risk Assessment
	Appendix G. Gypchek (Nucleopolyhedrovirus) Risk Assessment
	Appendix H. Disparlure Risk Assessment
	Appendix I. Diflubenzuron Risk Assessment
Volume IV	Appendix J. Tebufenozide Risk Assessment
	Appendix K. DDVP (Dichlorvos) Risk Assessment
	Appendix L. Gypsy Moth Risk Assessment
	Appendix M. Risk Comparison

All volumes can be viewed and downloaded at http://na.fs.fed.us/pubs/detail.cfm?id=5251.

The record of decision is a separate document published and available 30 days or longer after the notice of availability for the Final Supplemental Environmental Impact Statement is published in the Federal Register (40 CFR Part 1506.10).

Volume IV

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Gypsy Moth Management in the United States: *a cooperative approach*

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Control/Eradication Agents for the Gypsy Moth - Human Health and Ecological Risk Assessment for Tebufenozide (Mimic) Final Report

Prepared for:

USDA, Forest Service

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AEL	adverse-effect level
a.i.	active ingredient
BCF	bioconcentration factor
bw	body weight
CBI	confidential business information
CI	confidence interval
cm	centimeter
CNS	central nervous system
DAA	days after application
DAT	days after treatment
d.f.	degrees of freedom
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
ExToxNet	Extension Toxicology Network
F	female
FH	Forest Health
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FOIA	Freedom of Information Act
FQPA	Food Quality Protection Act
g	gram
ha	hectare
HQ	hazard quotient
IAA	indole-3-acetic acid
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
k_a	absorption coefficient
k_e	elimination coefficient
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
$K_{o/w}$	octanol-water partition coefficient
K_p	skin permeability coefficient
L	liter
lb	pound
LC_{50}	lethal concentration, 50% kill
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level
LOC	level of concern
m	meter

M	male
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
mM	millimole
MRID	Master Record Identification Number
MSDS	material safety data sheet
MW	molecular weight
NCAP	Northwest Coalition for Alternatives to Pesticides
NCI	National Cancer Institute
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
ppm	parts per million
RBC	red blood cells
RED	re-registration eligibility decision
RfD	reference dose
SERA	Syracuse Environmental Research Associates
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SRC	Syracuse Research Corporation
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
WCR	water contamination rate
WHO	World Health Organization
µ	micron

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

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

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

CONVERSION OF SCIENTIFIC NOTATION

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

EXECUTIVE SUMMARY

OVERVIEW

The use of tebufenozide to control the gypsy moth may result in adverse effects in nontarget *Lepidoptera* but there is little indication that humans or non-lepidopteran wildlife species will be impacted under normal conditions of use even at the highest application rate.

The only hazard quotient for humans that exceeds the level of concern (HQ of 1.5) involves the longer term consumption of contaminated vegetation. While the longer-term consumption of contaminated vegetation is probably not a likely scenario, it is a standard exposure scenario used in Forest Service risk assessments to consider the longer term consumption of food items such as berries that might be sprayed during the broadcast application of a pesticide. This risk assessment suggests that two applications at 0.08 lb/acre or more should be avoided in areas where members of the general public might consume contaminated fruits or other contaminated vegetation.

Tebufenozide is an insecticide that is effective in controlling populations of lepidopteran pests. No data, however, are available on toxicity to nontarget *Lepidoptera*. For this risk assessment, the assumption is made that nontarget *Lepidoptera* may be as sensitive to tebufenozide as target *Lepidoptera*. Thus, adverse effects in nontarget *Lepidoptera* would be expected after applications that are effective for the control of lepidopteran pest species.

There is no indication that short term exposures to tebufenozide will cause adverse effects in any terrestrial vertebrates or non-lepidopteran invertebrates even at the upper range of plausible exposures as well as accidental exposures. Similarly, adverse effects from longer terms exposures in birds and mammals appears to be unlikely under most conditions. In some extreme cases, exposures in some large mammals could exceed the NOEC but the but exposures would be below levels that have been associated with frank signs of toxicity. Adverse effects in aquatic species are not expected under normal conditions of use. In the case of a large accidental spill into a relatively small body of water, however, adverse effects could be expected in aquatic vertebrates, invertebrates, and plants.

PROGRAM DESCRIPTION

Mimic is a commercial formulation of tebufenozide, a synthetic chemical that acts like an invertebrate hormone that controls molting in insects and various terrestrial and aquatic invertebrates. While Mimic is specifically used by the USDA for the control of the Gypsy moth, tebufenozide is also used in the control of other lepidopteran pest species. Mimic is comprised of 23-25% tebufenozide and 75-77% inert ingredients. The inert ingredients consist of glycerol, related reaction products, alkylaryl polyether alcohol, glyceridic and canola oils, and water. Tebufenozide is relatively persistent in the environment and may be subject to bioconcentration. Although the compound is not highly mobile in soil, it may be transported by percolation or runoff from soil to ambient water. Potential concentrations of tebufenozide in ambient water depend largely on site specific conditions.

Ground and aerial applications of Mimic are permitted and both methods may be considered in USDA programs. The labeled application rates for tebufenozide range from 2 to 8 ounces of Mimic/acre, corresponding to tebufenozide application rates of 0.03 lbs/acre to 0.12 lbs/acre. Multiple applications of tebufenozide are permitted but the maximum annual application rate is 16 fl ounces/acre or 0.24 lb a.i./acre. The application rates for Mimic may vary among USDA programs – i.e., suppression, eradication, and Slow-the-Spread. For the current risk assessment, a range of application rates – i.e., 0.015 lb a.i./acre to 0.12 lb a.i./acre – are considered. All exposure assessments are conducted at the maximum application rate of 0.12 lb/acre, assuming two applications with a 3 day interval. This is essentially a worse-case scenario using a shortest interval between applications and two applications that reach the maximum annual application rate of 0.24 lb/acre.

HUMAN HEALTH RISK ASSESSMENT

Hazard Identification – A relatively detailed and consistent series of studies in mice, rats, and dogs indicates that the primary mechanism of tebufenozide toxicity in mammals involves effects on the blood. Tebufenozide does not appear to be carcinogenic and does not appear to cause birth defects. Nonetheless, the compound is associated with adverse reproductive effects in experimental mammals. Tebufenozide itself does not seem to be irritating to the skin or eyes. Mimic, however, appears to contain other constituents (inerts or adjuvants) that may cause skin or eye irritation.

As discussed in the exposure assessment, dermal absorption is the primary route of exposure for workers. Data regarding the dermal absorption kinetics of tebufenozide are not available in the published or unpublished literature. For this risk assessment, estimates of dermal absorption rates are based on quantitative structure-activity relationships. Although the lack of experimental data regarding dermal absorption of tebufenozide adds uncertainties to this risk assessment, the available data regarding the oral and dermal toxicity of tebufenozide are sufficient to suggest that the estimated dermal absorption rates are plausible.

The inhalation toxicity of tebufenozide is not well documented in the literature. The available studies indicate that tebufenozide induces irritant effects at very high exposure levels. Because inhalation exposure involving high concentrations of tebufenozide is implausible, the potential inhalation toxicity of the compound is not of substantial concern to this risk assessment.

Exposure Assessment – A standard set of exposure scenarios are presented for both workers and members of the general public. All exposure assessments are conducted at the maximum application rate for tebufenozide of 0.12 lb/acre using two applications with an application interval of three days. This cumulative application (0.24 lb a.i./acre) is the maximum application rate for a single season. This leads to the highest estimates of peak as well as longer term exposures.

For workers applying tebufenozide, three types of application methods are modeled: directed ground spray, broadcast ground spray, and aerial spray. Central estimates of exposure for

workers are approximately 0.002 mg/kg/day for aerial and backpack workers and about 0.003 mg/kg/day for broadcast ground spray workers. Upper ranges of exposures are approximately 0.02 mg/kg/day for broadcast ground spray workers and 0.01 mg/kg/day for backpack and aerial workers. All of the accidental exposure scenarios for workers involve dermal exposures and most of these accidental exposures lead to estimates of dose that are either in the range of or substantially below the general exposure estimates for workers. The one exception involves wearing contaminated gloves for one-hour. The upper range of exposure for this scenario is about 4 mg/kg/day.

For the general public, the range of acute exposures is from approximately 0.0000002 mg/kg associated with the lower range for the consumption of contaminated water from a stream by a child to 1.2 mg/kg associated with the upper range for consumption of contaminated water by a child after an accidental spill. Relatively high dose estimates are also associated with the direct spray of a child (about 0.4 mg/kg at the upper range of exposure) and for the consumption of fish after an accidental spill by members of the general public (0.2 mg/kg) and subsistence populations (0.9 mg/kg). Other acute exposure scenarios are associated with doses that are lower by at least an order of magnitude. For chronic or longer term exposures, the modeled exposures are much lower than for acute exposures, ranging from approximately 0.000000002 mg/kg/day (2 in 1 billionth of a mg/kg/day) associated with the lower range for the consumption of contaminated water to approximately 0.03 mg/kg/day associated with the upper range for consumption of contaminated fruit.

Dose-Response Assessment – Acute and chronic risk values are derived for tebufenozide. Following standard practices for USDA risk assessments, risk assessment values available from U.S. EPA are adopted directly unless there is a compelling basis for doing otherwise. When risk values are not available from U.S. EPA, the methods used by U.S. EPA are employed to derive surrogate values.

U.S. EPA has derived a chronic RfD for tebufenozide of 0.018 mg/kg/day. This chronic RfD is well-documented and is used directly for all longer term exposures to tebufenozide. This value is based on a NOAEL of 1.8 mg/kg/day in dogs and an uncertainty factor of 100 – two factors of 10 for interspecies and intraspecies variability. Because of the low acute toxicity of tebufenozide, the U.S. EPA has not derived an acute RfD but has identified an acute NOAEL of 1000 mg/kg/day from reproduction studies in both rats and rabbits involving 10 to 13 day exposure periods. This NOAEL is the basis for a surrogate acute RfD of 10 mg/kg using an uncertainty factor of 100 as in the chronic RfD. This surrogate acute RfD is applied to all incidental or accidental exposures that involve an exposure period of 1 day.

Risk Characterization – At the maximum application rate considered in this risk assessment, two applications at 0.12 lb/acre spaced three day apart, there is little indication that adverse effects on human health are likely. Based on central estimates of exposure – those that might be considered typical and expected – hazard quotients including workers and members of the general public range from 0.00003 to 0.03, below a level of concern by factors of about 30 to

33,000. At the upper range of plausible exposures, the hazard quotient for ground spray workers reaches a level of concern – i.e., a hazard quotient of 1. For members of the general public, the upper range of exposure leads to a hazard quotient of 1.5 for the longer-term consumption of contaminated vegetation for two applications at 0.12 lb/acre. Because of the linear relationship between exposure and application rate, two applications at 0.08 lb/acre would reach but not exceed a level of concern. With a single application at the maximum rate of 0.12 lb/acre, the hazard index is 0.8, below the level of concern. While the longer-term consumption of contaminated vegetation is probably not a likely scenario, it is a standard exposure scenario used in Forest Service risk assessments to consider the longer term consumption of food items such as berries that might be sprayed during the broadcast application of a pesticide. This risk assessment suggests that two applications at 0.08 lb/acre or more should be avoided in areas where members of the general public might consume contaminated fruits or other contaminated vegetation.

ECOLOGICAL RISK ASSESSMENT

Hazard Identification **–** The toxicity of tebufenozide is well characterized in experimental mammals, birds, terrestrial invertebrates, and aquatic animals. Nonetheless, given the very large number of species in the environment which could be exposed to tebufenozide, toxicity data are available on relatively few species.

The most sensitive effects in wildlife mammalian species will probably be the same as those in experimental mammals (i.e., effects on the blood). At higher doses, tebufenozide was associated with impaired reproductive performance in experimental mammals, and this effect is also considered quantitatively in this risk assessment. Potential reproductive effects are also of concern for birds, although there are inconsistencies in the available experimental data. The available literature includes a reproduction study investigating effects in mallard ducks and two reproduction studies investigating effects in bobwhite quail. In one of the quail studies, dietary concentrations of 300 and 1000 ppm caused reproductive effects. These effects were not observed in that study at 100 ppm or in the more recent quail study or in the study on mallard ducks. A field study on the effects of tebufenozide on reproductive performance in birds noted trends that were statistically insignificant but suggestive of adverse reproductive effects in a warbler species. Thus, consistent with the interpretation by the U.S. EPA, reproductive effects in both mammals and birds are considered endpoints of concern in this risk assessment.

The mechanism of action of tebufenozide in target insects is relatively well understood. Tebufenozide mimics the action of the invertebrate hormone, 20-hydroxyecdysone, which controls molting. The effectiveness of tebufenozide in mimicking 20-hydroxyecdysone activity, however, appears to vary markedly among orders and species of invertebrates. In general, moths are sensitive to tebufenozide but other insects are much less sensitive.

There are no bioassays regarding the toxicity of tebufenozide to terrestrial plants or terrestrial microorganisms in the literature. There are a number of field studies and field simulation studies available on tebufenozide and effects that might be associated with toxicity to plants or soil

microorganisms have not been noted.

The acute toxicity of tebufenozide to aquatic animals is relatively low, with acute LC_{50} values ranging from 2.2 to 6.5 mg/L for fish and 0.3 to 3.8 mg/L for aquatic invertebrates. Nonetheless, much lower concentrations of tebufenozide may cause reproductive effects in fish (0.048 mg/L) and aquatic invertebrates (0.0053 mg/L).

Exposure Assessment – As in the human health risk assessment, most exposure assessments used in the ecological risk assessment are based on two applications spaced 3 days apart at an application rate of 0.12 lb/acre. Two sets of exposure assessments are given for scenarios involving the longer-term consumption of contaminated vegetation: one for a single application at 0.12 lb/acre and another for two applications spaced 3 days apart at an application rate of 0.12 lb/acre.

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. For tebufenozide, the highest acute exposure for a terrestrial vertebrate is associated with a fish-eating bird and could reach up to about 85 mg/kg. Exposures anticipated from the consumption of contaminated vegetation by terrestrial animals range from central estimates of about 0.15 mg/kg for a small mammal consuming fruit to about 3 mg/kg for a large bird with upper ranges of about 0.4 mg/kg for a small mammal and 9 mg/kg for a large bird. The consumption of contaminated water leads to much lower levels of exposure. A similar pattern is seen for chronic exposures. Estimated longer-term daily doses for the a small mammal from the consumption of contaminated vegetation at the application site are in the range of about 0.000002 mg/kg/day to 0.08 mg/kg/day. Large birds feeding on contaminated vegetation at the application site could be exposed to much higher concentrations, ranging from about 0.015 mg/kg/day to 11 mg/kg/day. The upper ranges of exposure from contaminated vegetation far exceed doses that are anticipated from the consumption of contaminated water, which range from about 0.0000003 mg/kg/day to 0.0002 mg/kg/day for a small mammal.

Exposure to aquatic organisms is based on essentially the same information used to assess the exposure to terrestrial species from contaminated water. The peak estimated concentration of tebufenozide in ambient water is 10 (0.005 to 40) µg/L after two applications of 0.12 lb/acre spaced three days apart. For longer-term exposures, the corresponding longer term concentrations in ambient water are estimated at about 0.004 (0.00002 to 0.01) µg/L.

Dose-Response Assessment – The available toxicity data support separate dose-response assessments in six classes of organisms: terrestrial mammals, birds, nontarget terrestrial invertebrates, fish, aquatic invertebrates, and aquatic algae. Different units of exposure are used for different groups of organisms depending on how exposures are likely to occur and how the available toxicity data are expressed.

Tebufenozide is relatively non-toxic to mammals and birds. For mammals, the toxicity values

used in the ecological risk assessment are identical to those used in the human health risk assessments: an acute NOAEL for reproductive toxicity of 1000 mg/kg and a chronic NOAEL of 1.8 mg/kg/day based on effects on the blood. For birds, the acute NOAEL for tebufenozide is taken as 2150 mg/kg from an acute oral study in which the dose was administered in capsules for 21-days. The longer term NOAEL is taken as 15 mg/kg/day from a standard reproduction study in bobwhite quail.

For terrestrial invertebrates, three types of data are used to characterize risks: a contact bioassay in the honey bee, a soil bioassay in earthworms, and field studies in which population level effects were monitored in insects. The standard contact bioassay in honey bees indicates an NOEC of 2500 mg/kg bw, comparable to the acute toxicity values in mammals and birds. The earthworm bioassay indicates a NOEC of 1000 mg/kg soil. The available field studies indicate that tolerant insect species are not affected by application rates up to 0.24 lb/acre. The true NOEC may be higher – i.e., an LOEC has not been identified for tolerant species of terrestrial insects. Conversely, application rates as low as 0.03 lb/acre have been shown to adversely affect sensitive non-target insects, primarily *Lepidoptera*. A NOEC for sensitive species has not been identified.

Acute toxicity values for aquatic species indicate relatively little difference between fish and aquatic invertebrates. For fish, the acute NOEC values are 0.39 mg/L and 1.9 mg/L for sensitive and tolerant species, respectively. For invertebrates, the corresponding acute NOEC values are 0.12 mg/L and 0.82 mg/L. Differences between fish and invertebrates are difficult to assess in terms of longer-term toxicity. For fish, data are available on only a single species, the fathead minnow, and only a LOAEL of 0.048 mg/L is available. For invertebrates, longer-term NOEC values of 0.0035 mg/L and 0.029 mg/L are used for sensitive and tolerant species. Toxicity values for aquatic plants are taken as 0.077 mg/L for sensitive species and 0.64 mg/L for tolerant species, somewhat below the acute NOEC values in fish and aquatic invertebrates. Because of the short life-cycle of individual algal cells, the relatively short-term bioassays in algae (i.e., 96 to 120 hours) are applied to both acute and longer-term concentrations for the characterization of risk.

Risk Characterization – The use of tebufenozide to control the gypsy moth may result in adverse effects in nontarget *Lepidoptera* but there is little indication that other species will be impacted under normal conditions of use even at the highest application rate. Tebufenozide is an insecticide that is effective in controlling populations of lepidopteran pests. No data, however, are available on toxicity to nontarget *Lepidoptera*. For this risk assessment, the assumption is made that nontarget *Lepidoptera* may be as sensitive to tebufenozide as target *Lepidoptera*. Thus, adverse effects in nontarget *Lepidoptera* would be expected after applications that are effective for the control of lepidopteran pest species.

There is no indication that short term exposures to tebufenozide will cause direct adverse effects in any terrestrial vertebrates or non-lepidopteran invertebrates even at the upper range of plausible exposures as well as accidental exposures. Similarly, direct adverse effects from longer

term exposures in birds and mammals appear to be unlikely under most conditions. Effects on birds due to a decrease in available prey – i.e., terrestrial invertebrates – may be plausible. In some extreme cases, exposures in some large mammals could exceed the NOEC but the exposures would be below levels that have been associated with frank signs of toxicity. Adverse effects in aquatic species are not expected under normal conditions of use. In the case of a large accidental spill into a relatively small body of water, however, adverse effects could be expected in aquatic vertebrates, invertebrates, and plants.

1. INTRODUCTION

The USDA uses Mimic, a commercial formulation of tebufenozide, to control infestations of the Gypsy Moth. This risk assessment is an update to a risk assessment prepared for the USDA Forest Service in 2000 (SERA 2000) and is intended to support an assessment of the environmental consequences of using Mimic in USDA programs for the control of the gypsy moth.

For the most part, the risk assessment methods used in this document are similar to those used in risk assessments previously conducted for the Forest Service as well as risk assessments conducted by other government agencies. Four chapters, including the introduction, program description, risk assessment for human health effects, and risk assessment for ecological effects or effects on wildlife species comprise the main body of this document. Each of the two risk assessment chapters has four major sections, including an identification of the hazards associated with Mimic, an assessment of potential exposure to the product, an assessment of the dose-response relationships, and a characterization of the risks associated with plausible levels of exposure. These sections incorporate the basic steps recommended by the National Research Council of the National Academy of Sciences (NRC 1983) for conducting and organizing risk assessments.

This is a technical support document, and it addresses some specialized technical areas. Nevertheless, an effort was made to ensure that the document can be understood by individuals who do not have specialized training in the chemical and biological sciences. Certain technical concepts, methods, and terms common to all parts of the risk assessment are described in plain language in a separate document (SERA 2001). The general technical terms used in this document are defined in an environmental glossary available at www.sera-inc.com. Some of the more complicated terms and concepts are defined, as necessary, in the text.

There are no detailed reviews regarding the toxicity of tebufenozide or Mimic in the published literature. Risk assessments for human health and ecological effects were conducted by the U.S. EPA (1999a,b,c,d,e). The registrant for Mimic at that time, Rohm and Haas, also prepared a series of risk assessments and other evaluations on Mimic (Hawkins 1998; Hazelton and Quinn 1994; Kaminski 1997; Keller 1994, 1996a, 1998; Keller and Brown 1998a,b; Quinn and Hazelton 1997). These unpublished documents were obtained and reviewed in the preparation of this Forest Service risk assessment.

Because of the preponderance of unpublished relevant data in U.S. EPA files, a complete search of the U.S. EPA files was conducted in the preparation of this risk assessment. Full text copies of the most relevant studies [n=107] were kindly provided by the U.S. EPA Office of Pesticide Programs. The studies were reviewed, and synopses of the most relevant studies are included in the appendices to this document.

The information presented in the appendices and the discussions in chapters 2, 3, and 4 of the

risk assessment are intended to be detailed enough to support a review of the risk analyses; however, they are not intended to be as detailed as the information generally presented in Chemical Background documents or other comprehensive reviews. Almost no risk estimates presented in this document are given as single numbers. Usually, risk is expressed as a central estimate and a range, which is sometimes very large. Because of the need to encompass many different types of exposure as well as the need to express the uncertainties in the assessment, this risk assessment involves numerous calculations. Most of the calculations are relatively simple, and the very simple calculations are included in the body of the document. Some of the calculations, however, are cumbersome. For those calculations, worksheets are included as an attachment to the risk assessment. The worksheets provide the detail for the estimates cited in the body of the document. The worksheets are divided into the following sections: general data and assumptions, chemical specific data and assumptions, exposure assessments for workers, exposure assessments for the general public, and exposure assessments for effects on nontarget organisms. The worksheets for tebufenozide are contained in an EXCEL workbook and are included as Supplement 1 to this risk assessment. SERA (2004a) contains documentation for the use of these worksheets.

2. PROGRAM DESCRIPTION

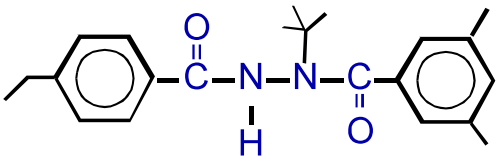
2.1. OVERVIEW

Mimic is a commercial formulation of tebufenozide, a synthetic chemical that acts like an invertebrate hormone that controls molting in insects and various terrestrial and aquatic invertebrates. While Mimic is specifically used by the USDA for the control of the Gypsy moth, tebufenozide is also used in the control of other lepidopteran pest species. Mimic is comprised of 23-25% tebufenozide and 75-77% inert ingredients. The inert ingredients consist of glycerol, related reaction products, alkylaryl polyether alcohols, glyceridic and canola oils, and water. Additional specific information on the inerts was reviewed in the preparation of this risk assessment. The specific chemical identity of these inerts cannot be provided in this public document. Tebufenozide is relatively persistent in the environment and may be subject to bioconcentration. Although the compound is not highly mobile in soil, it may be transported by percolation or runoff from soil to ambient water. Potential concentrations of tebufenozide in ambient water depend largely on site specific conditions.

Ground and aerial applications of Mimic are permitted and both methods may be considered in USDA programs. The labeled application rates for tebufenozide range from 2 to 8 ounces of Mimic/acre, corresponding to tebufenozide application rates of 0.03 lbs/acre to 0.12 lbs/acre. Multiple applications of tebufenozide are permitted but the maximum allowable cumulative amount applied is is 16 fl ounces/acre or 0.24 lb a.i./acre. The application rates for Mimic may vary among these USDA programs – i.e., suppression, eradication, and slow the spread. For the current risk assessment, the range of labeled application rates – i.e., 0.015 lb a.i./acre to 0.12 lb a.i./acre – are considered. All exposure assessments are conducted at the maximum application rate of 0.12 lb/acre, assuming two applications with a 3 day interval. This is essentially a worse-case scenario using a shortest interval between applications and two applications that reach the maximum annual application rate of 0.24 lb/acre. The consequences of using lesser rates are considered in the risk characterization for human health (Section 3.4) and ecological effects (Section 4.4).

2.2. CHEMICAL DESCRIPTION AND COMMERCIAL FORMULATIONS

Mimic 2LV, hereafter referred to simply as Mimic, is an insecticide initially registered by Rohm and Haas and currently registered by Dow AgroSciences (C&P Press 2004). The active ingredient (a.i.) in Mimic is tebufenozide, the common name for 3,5-dimethyl-, (1,1-dimethylethyl)-2-(4-ethylbenzoyl) hydrazide benzoic acid:



As detailed in Section 4.1.2.3, tebufenozide mimics the action of the invertebrate hormone 20-hydroxyecdysone. This hormone controls molting in insects and various terrestrial and aquatic invertebrates. While Mimic is specifically used by USDA for the control of the Gypsy moth, tebufenozide is effective in the control of other lepidopteran pest species.

Selected chemical and physical properties of tebufenozide are summarized in Table 2-1, and the physical and chemical properties that are directly used in this risk assessment are presented in worksheet B03. Dow AgroSciences also provides two other formulations, Confirm 2F and Confirm TO, that contains tebufenozide as the active ingredient (C&P Press 2004).

Mimic is comprised of 23-25% tebufenozide and 75-77% inert ingredients. The inert ingredients consist of glycerol, related reaction products, alkylaryl polyether alcohols, glyceridic and canola oils (not otherwise specified), and water. The specific identity of the alkylaryl polyether alcohols as well as the amounts of each of the other inert ingredients is considered a trade secret proprietary to Dow AgroSciences. Hence, this information is not identified on the product labels or material safety data sheets (C&P Press 1999). Information about the impurities in technical grade tebufenozide were submitted to the U.S. EPA by the initial registrant (Kelly 1992; Patel 1998) and this information was reviewed in the preparation of this risk assessment. Although additional specific information on the inerts cannot be provided in this public document, the potential impact of inert ingredients and product impurities is considered in Section 3.1.9. Spray adjuvants are not recommended for use with Mimic and are not given further consideration in this risk assessment.

The environmental fate and transport of tebufenozide is relatively well characterized in studies conducted as part of the registration process for this pesticide (Hawkins 1992, 1993, 1994, 1996, 1998) as well as in series of studies conducted by the Canadian Forest Service (Sundaram 1994a,b, 1995, 1996, 1997a, 1997b; Sundaram et al. 1996ab, 1997a, 1997b). Pertinent information about the environmental fate and transport of tebufenozide is provided in Table 2-1. Additional detailed on environmental fate and transport are discussed in the exposure assessments for human health effects (Section 3.2) as well as ecological effects (Section 4.2). Briefly, tebufenozide is relatively persistent in the environment and may be subject to bioconcentration. Although the compound is not highly mobile in soil, it may be transported by percolation, sediment, or runoff from soil to ambient water. Potential concentrations of tebufenozide in ambient water depend largely on site specific conditions.

2.3. APPLICATION METHODS

The product label for Mimic indicates that ground or aerial applications are permitted, and both methods may be considered for use by the USDA. Supplemental labels indicating further restrictions on ground or aerial applications were not located (C&P Press 1999).

The most common method for ground application of Mimic is hydraulic sprayers, mist blowers, or air blast sprayers (broadcast foliar). The spray equipment is typically mounted on tractors or trucks used to apply the insecticide on either side of the roadway. Usually, about 8 acres are

treated in a 45-minute period (approximately 11 acres/hour). Special truck-mounted spray systems may be used to treat up to 12 acres in a 35-minute period with approximately 300 gallons of insecticide mixture (approximately 21 acres/hour and 510 gallons/hour) (USDA 1989b, p 2-9 to 2-10).

In some instances, directed foliar applications may be used. In selective foliar applications, the sprayer or container containing the pesticide is carried by backpack and 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 acres/hour with a plausible range of 0.25-1.0 acre/hour.

In aerial applications, Mimic 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 10 acres may be treated per minute (Reardon 2000).

2.4. MIXING AND APPLICATION RATES

The labeled application rates for tebufenozide range from 2 to 8 ounces of Mimic/acre, corresponding to tebufenozide application rates of 0.03 lbs/acre to 0.12 lbs/acre. This range of application rates is recommended for the control of Gypsy moth and several other lepidopteran pest species. The highest recommended application rate for any species is 8 ounces of Mimic/acre or 0.12 lb tebufenozide per acre. This is the only application rate recommended for the control of the pine tip moth. Application rates from 4 to 8 ounces of Mimic per acre are recommended on the label for gypsy moth. The maximum amount of Mimic that may be applied per year is 16 fl ounces/acre or 0.24 lb a.i./acre (C&P Press 2004).

Commercial formulations of tebufenozide are diluted with water prior to application. In ground applications, application volumes of 50 gallons per acre are recommended for hydraulic ground sprayers and a minimum of 10 gallons per acre is recommended for mist blowers or air blast sprayers. For aerial applications, a minimum of 0.5 gallon per acre is recommended. As specified on the product label, uniform coverage is essential for efficacy and higher spray volumes are recommended for large trees, dense stands, and/or heavy infestations (C&P Press 2004).

The USDA has adopted various intervention strategies that are roughly categorized as suppression, eradication, and Slow-the-Spread (Liebhold and McManus 1999). These programs may be conducted by either the USDA Forest Service or the Animal and Plant Health Inspection Service (APHIS). Suppression efforts are conducted in areas of well established gypsy moth infestations to combat or interdict periodic gypsy moth population outbreaks. Eradication efforts are intended to completely eliminate gypsy moth populations in areas where new populations of the gypsy moth are found. Slow-the-Spread, as the name implies, is a program to reduce the

expansion of gypsy moth populations from areas of established populations to adjacent non-infested areas.

The application rates for Mimic may vary among these USDA programs. For the USDA Forest Service, the typical application rates will range from 0.015 to 0.06 lb a.i. per acre. A single application is used in suppression programs and two to three applications may be made in eradication programs. Mimic as well as other formulations of tebufenozide may be reapplied. The interval between applications in Forest Service programs will generally be 3 to 10 days. The Forest Service may consider using the maximum application rate of 0.12 lb a.i./acre in some instances (Cook 2004). In eradication programs, APHIS will use an application rate of 0.06 lb a.i. per acre. Two applications may be made with an application interval of 7 to 10 days.

For the current risk assessment, the range of labeled application rates – i.e., 0.015 lb a.i./acre to 0.12 lb a.i./acre – are considered. All exposure assessments will be conducted at the maximum application rate of 0.12 lb/acre, assuming two applications with a 3 day interval. This is essentially a worst-case scenario using a shortest interval between applications and two applications that reach the maximum annual application rate of 0.24 lb/acre. The consequences of using lesser rates are considered further in the risk characterization for human health (Section 3.4) and ecological effects (Section 4.4).

Mimic is diluted prior to application. In this risk assessment, the extent to which Mimic is diluted prior to application primarily influences dermal and direct spray scenarios, both of which depend on the 'field dilution' (i.e., the concentration of tebufenozide in the applied spray). Invariably, the higher the concentration of tebufenozide, the greater the risk. For this risk assessment, the lowest dilution is taken at 0.5 gallon/acre, the minimum recommended for aerial applications. The highest dilution (i.e., that which results in the lowest risk) is based on 50 gallons of water per acre, the highest application volume specifically recommended on the product label (C&P Press 2004). The central estimate is taken as 5 gallons of water per acre, the geometric mean of the range. Detailed calculations of field dilution rates are provided in worksheet B01, and the calculations following worksheet B01 and the values used in various exposure assessments are summarized in worksheet B02.

2.5. USE STATISTICS

Neither Mimic nor other pesticides containing tebufenozide have been used previously by the USDA in full scale control programs. Consequently past use statistics that might reflect the amounts of tebufenozide that may be used in USDA programs are not available. Experimental programs have been conducted by the USDA in the northeast and have involved the treatment of experimental plots ranging from 16 to 135 acres (Reardon 2000).

Tebufenozide was used extensively as a pest control agent on cotton. In 1992, the most recent year for which data are available, 42,104 lbs were used for that purposes. As illustrated in Figure 2-1, all of the tebufenozide applied to cotton in1992 was used in Texas and Mississippi (USGS 1998).

Tebufenozide is used in Canada at an application rate of 0.07 kg a.i./ha or 0.062 lb a.i./acre to control spruce budworms. In 1994, only 400 acres were treated; however, in 1997, 14,875 acres were treated (Canadian Council of Forest Ministers 1999), and the amount of tebufenozide used is calculated as 922.25 lbs [14,875 acres × 0.062 lb a.i./acre].

3. HUMAN HEALTH RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION

3.1.1. Overview

A relatively detailed and consistent series of studies in mice, rats, and dogs indicates that the primary mechanism of tebufenozide toxicity in mammals involves hematological effects, specifically the formation of methemoglobin. Tebufenozide does not appear to be carcinogenic and does not appear to cause birth defects. Nonetheless, the compound is associated with adverse reproductive effects in experimental mammals. Tebufenozide itself does not seem to be irritating to the skin or eyes. Mimic, however, appears to contain other constituents (inerts or adjuvants) that may cause skin or eye irritation.

As discussed in the exposure assessment, dermal absorption is the primary route of exposure for workers. Data regarding the dermal absorption kinetics of tebufenozide are not available in the published or unpublished literature. For this risk assessment, estimates of dermal absorption rates are based on quantitative structure-activity relationships. The estimated dermal absorption rates are used in turn to estimate the amounts of tebufenozide that might be absorbed by workers. Then, those estimates are used with the available dose-response data to characterize risk. Although the lack of experimental data regarding dermal absorption of tebufenozide adds uncertainties to this risk assessment, the available data regarding the oral and dermal toxicity of tebufenozide are sufficient to suggest that the estimated dermal absorption rates are plausible.

The inhalation toxicity of tebufenozide is not well documented. Irritant effects have been noted in laboratory studies involving exposures to very high concentrations of tebufenozide in air. Because inhalation exposure involving high concentrations of tebufenozide is implausible under normal field conditions, the potential inhalation toxicity of the compound is not of substantial concern to this risk assessment.

3.1.2. Mechanism of Action

In mammals, tebufenozide is known to damage hemoglobin, a key component of blood, through the formation of methemoglobin. This is highly relevant to the human health risk assessment because effects on the blood are the basis for the U.S. EPA RfD for tebufenozide (Section 3.3).

Hemoglobin is the component in red blood cells that is responsible for transporting oxygen throughout the body. If this function is impaired, either because of damage to hemoglobin or lack of oxygen in the air, serious adverse effects (i.e., equivalent to suffocation) can occur. The formation of both methemoglobin and sulfhemoglobin can cause such impairment and lead to the formation of methemoglobinemia and sulfhemoglobinemia, respectively. Methemoglobin is formed by the oxidation of the heme iron in hemoglobin from the ferrous (Hb^{++}) to the ferric state ($MetHb^{+++}$) (Bradberry 2003; Smith 1996). Heme group oxidation occurs spontaneously and accounts for approximately 2% of the hemoglobin in normal individuals. Methemoglobin is reduced (restored to its natural state) by a set of enzymes referred to as methemoglobin reductases. Some individuals are deficient in NADH-dependent methemoglobin reductase, in

which case as much as 50% of their blood pigment may exist as methemoglobin. Newborns are also deficient in NADH-methemoglobin reductase.

While tebufenozide displays other types of toxicity, as discussed in the following subsections, the formation of methemoglobin is the only mechanisms of toxicity that has been clearly identified.

3.1.3. Kinetics and Metabolism

3.1.3.1. Pharmacokinetic Studies – The pharmacokinetics of tebufenozide have been studied in rats after oral doses of 3 or 250 mg/kg of ^{14}C-labeled tebufenozide (Struble and Hazelton 1992). Tebufenozide was rapidly absorbed and excreted. Concentrations of tebufenozide in blood were not linearly related to dose. Concentrations of tebufenozide in the blood were only about 4 to 6 times those in the low dose. While absorption rates are not calculated in Struble and Hazelton (1992), this pattern suggests a less rapid absorption rate in the high dosed animals or a saturation of critical pathways involving absorption. About 75% to 99% was excreted in the feces during the first 24 hours with virtually complete excretion by 48 hours after dosing. In the blood, most of the radioactivity was associated with blood cells rather than plasma – i.e., blood to plasma ratios of 10:1 to 15:1.

3.1.3.1. Dermal Absorption Rates – As detailed further in Section 3.2.2.2, two types of dermal exposure scenarios are considered in this risk assessment: those involving direct contact with a solution of the herbicide (e.g., immersion) and those associated with accidental spills of the herbicide onto the surface of the skin.

As detailed in SERA (2001), dermal exposure scenarios involving immersion or prolonged contact with chemical solutions use Fick's first law and require an estimate of the permeability coefficient, K_p, expressed in cm/hour. Using the method recommended by U.S. EPA (1992), the estimated dermal permeability coefficient for tebufenozide is 0.013 cm/hour with a 95% confidence interval of 0.0066-0.025 cm/hour. These estimates are used in all exposure assessments that are based on Fick's first law. For exposure scenarios like direct sprays or accidental spills, which involve deposition of the compound on the skin's surface, dermal absorption rates (proportion of the deposited dose per unit time) rather than dermal permeability rates are used in the exposure assessment. The estimated first-order dermal absorption coefficient is 0.0032 hour^{-1} with 95% confidence intervals of 0.0012-0.0082 hour^{-1}. The calculations for these estimates are presented in Appendix 1. Note that the values for both dermal permeability and the first order dermal absorption rates are rounded to two significant figure in Table A1-5 of Appendix 1 and these values are entered into Worksheet A03 and used in all scenarios involving dermal exposures for both workers (Worksheet Series C) and the general public (Worksheet Series D).

There are no experimental data regarding the absorption of tebufenozide by humans. Wederbrand and Potter (1993) report that a proportion of 0.05 of a dermal dose of tebufenozide was absorbed by rats after 10 hours. The ^{14}C-tebufenozide was dissolved in a solution that approximated the 2F formulation – i.e., Confirm. While the specific ingredients in the

formulation are specified in a confidential appendix to this study, these ingredients (other than the general description given in Section 2) cannot be disclosed in this risk assessment. Taking 0.05 as the absorbed dose, the first-order dermal absorption coefficient would be about [k = -ln(1-0.05)/10 hours = 0.005 per hour]. This is very close to the estimate of 0.0032 $hour^{-1}$ given above. Thus, at least for short term exposures, the available data on absorption kinetics in rats are consistent with the estimate of the human first-order dermal absorption rate. Consequently, the lack of human data regarding the dermal absorption rate of tebufenozide adds relatively little uncertainty to this risk assessment. In addition, the available dermal toxicity data are adequate to address this uncertainty to some extent (Section 3.1.12.).

3.1.4. Acute Toxicity

Information regarding the acute oral toxicity of tebufenozide is summarized in Appendix 2. All of the available studies are standard bioassays conducted as part of the registration process for Mimic. Tebufenozide has a very low order of acute toxicity to mammals. Single oral gavage doses of 2000 mg/kg caused no observable signs of toxicity in mice or rats (Hazleton and Quinn 1995b; Swenson et al. 1994). Mimic, the commercial formulation of tebufenozide covered in this risk assessment, caused no signs of toxicity at doses of up to 5 g/kg or 5000 mg/kg (Parno and Gingrich 1994b). Mimic contains 23-25% tebufenozide by weight (see section 2), which corresponds to tebufenozide doses of about 1250 mg/kg body weight. As discussed in section 3.1.9.3, Mimic contains inert ingredients, the identity of which cannot be disclosed in this document. The lack of evidence that Mimic is toxic at a dose of 5000 mg/kg is consistent with the acute toxicity data on tebufenozide. Although this observation cannot be overly interpreted, it does at least suggest that the inerts in Mimic do not have a high order of acute oral toxicity.

3.1.5. Subchronic or Chronic Systemic Toxic Effects

Information on the subchronic and chronic oral toxicity of tebufenozide is summarized in Appendix 2. Like the acute studies, all of these studies were conducted as part of the registration process.

Appendix 2 summarizes subchronic studies in mice, rats, and dogs, with exposure durations ranging from 2 weeks to 90 days. The most consistently observed effects are related to the formation of methemoglobin, which can lead to decreases in red blood cell volume due to the destruction of the red blood cells (i.e., hemolytic anemia).

Methemoglobin induction involves the chemical oxidation of the heme iron in hemoglobin from the ferrous (Hb++) to the ferric state (MetHb+++), resulting in the inability of hemoglobin to combine reversibly with oxygen (Smith 1996). Heme group oxidation occurs spontaneously and accounts for approximately 2% of the hemoglobin in normal individuals. Methemoglobin is reduced (restored to its natural state) by a set of enzymes referred to as methemoglobin reductases. The most common methemoglobin reductase is dependent on NADH. Some individuals are deficient in NADH-dependent methemoglobin reductase, in which case, as much as 50% of their blood pigment may exist as methemoglobin. Newborns are also deficient in NADH-methemoglobin reductase. Aromatic amines are known to induce methemoglobinemia,

most likely by the formation of N-hydroxy metabolites (Smith 1996).

As discussed in section 3.3.2, methemoglobin formation and other effects on blood are the most sensitive endpoints for tebufenozide and is the basis for the U.S. EPA RfD for this compound. In test animals, specific changes in hematological parameters included decreases red blood cell count, mean cell volume, reticulocyte counts, methemoglobin, the incidence of Heinz bodies, and platelet counts as well as increases in spleen weight. The quantitative dose-response relationships for this effect are discussed further in section 3.3. Increased liver weight also was observed in three animal species [mice and rats (Osheroff 1991a,b), dogs (Clay 1992)]. This effect may be secondary to the formation of methemoglobin, which increases the destruction of red blood cells in the liver (Richards 1992a,b). Theoretically, increased liver weight may be observed as the result of enzyme induction in which a compound will induce enzymes that are associated with its own metabolism. This induction can lead to an increase in total liver weight and is often regarded as an adaptive rather than toxic response (Moslen 1996).

The chronic toxicity of tebufenozide was assayed in dogs (Richards 1992a,b), mice (Trutter 1992a,b) and rats (Trutter 1992c). As in the subchronic studies, signs of hemolytic anemia were observed in all three species.

3.1.6. Effects on Nervous System

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

In a standard assay for neurotoxicity, no signs of toxicity were noted in rats after single oral doses up to 2000 mg/kg (Swanson et al. 1994). In addition, signs of neurotoxicity have not been noted in a large number of acute and chronic toxicity studies (Appendices 2 and 3).

3.1.7. Effects on Immune System

Immunotoxicants are chemical agents that disrupt the function of the immune system. Two general types of effects, suppression and enhancement, may be seen and both of these are generally regarded as adverse. Agents that impair immune responses (*immune suppression*) enhance susceptibility to infectious diseases or cancer. Enhancement or *hyperreactivity* can give rise to *allergy* or hypersensitivity, in which the immune system of genetically predisposed individuals inappropriately responds to chemical or biological agents (e.g., plant pollen, cat dander, flour gluten) that pose no threat to other individuals or *autoimmunity*, in which the immune system produces antibodies to self components leading to destruction of the organ or tissue involved.

There is very little direct information on which to assess the immunotoxic potential of tebufenozide. The only studies specifically related to the effects of tebufenozide on immune function are skin sensitization studies (Section 3.1.11). While the studies by Anderson and Shuey (1994) and Glaza (1993) indicate that tebufenozide is not a skin sensitizer, this provides no information useful for directly assessing the potential for tebufenozide to suppress or otherwise disrupt immune function.

Nonetheless, the toxicity of tebufenozide has been examined in numerous acute, subchronic, and chronic bioassays. Although many of these studies did not focus on the immune system, changes in the immune system (which could potentially be manifest as increased susceptibility to infection compared to controls) were not observed in any of the available long-term animal studies (Appendix 2). 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 (Durkin and Diamond 2002). None of these effects have been noted in any of the longer term toxicity studies on tebufenozide (Appendix 2).

3.1.8. Effects on Endocrine System

The *endocrine system* 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. Adverse effects on the endocrine system can result in abnormalities in growth and development, reproduction, body composition, homeostasis (the ability to tolerate various types of stress), and behavior.

There is no indication that tebufenozide causes endocrine disruption in experimental mammals. Tebufenozide showed no activity in an *in vitro* test system (human estrogen receptor cDNA in the yeast, *Saccharomyces cerevisae*) for the human estrogen receptor (Cress 1996). In addition,

standard subchronic, chronic and reproductive toxicity studies (Section 3.1.9) provide no basis for asserting that any signs of overt toxicity are related to changes in endocrine function in mammals.

3.1.9. Reproductive and Teratogenic Effects

Tebufenozide was tested for its ability to cause birth defects (i.e., teratogenicity) as well as its ability to cause reproductive impairment. All of these studies are discussed in Appendix 2. Like the acute, subchronic, and chronic studies, all of the reproductive and developmental studies are unpublished and were conducted in support of the registration of this compound.

Teratogenicity studies usually entail gavage administration to pregnant rats or rabbits on specific days of gestation. Two such studies were conducted on tebufenozide: one in rats (Hoberman 1991) and one in rabbits (Swenson and Solomon 1992). No signs of teratogenicity or fetal toxicity were noted in either study. In the rat study, decreased weight gain was observed in dams treated with the highest dose (1000 mg/kg). Even at this dose, however, developmental effects were not observed.

Another type of reproduction study involves exposing more than one generation of the test animal to the compound. In other words, both the parent animals and the offspring are exposed to the substance. Two such studies (Aso 1995; Danberry et al. 1993) were conducted on tebufenozide. In the study by Aso (1995), signs of toxicity to the blood were observed in both male and female adult rats at dietary concentrations of 200 and 2000 ppm but not at a dietary concentration of 25 ppm. For offspring, no effects were observed at dietary concentrations of 25 or 200 ppm; however, treatment with 2000 ppm caused decreases in body weight. At the dietary concentration of 2000 ppm, the estimated dose levels were 126.0 mg/kg/day for males and 143.2 mg/kg/day for females (U.S. EPA 1999b). In the rat study by Danberry et al. (1993), no reproductive effects were observed at a dietary concentration of 150 ppm (≈ 12 mg/kg bw). At 2000 ppm (≈ 160 mg/kg bw), however, there was an increased incidence of mortality among females during delivery (P2), an increase in gestation length (P2), a decrease in the mean number of implantation sites per female (P2), and an increased incidence of pregnant females that did not deliver (P1 and P2).

As discussed further in section 4, there is concern for potential reproductive effects in birds. Based on a dietary study in quail (Beavers et al. 1993b), dietary concentrations of 300 or 1000 ppm, corresponding to estimated doses of 45 or 150 mg/kg bw, were associated with decreases in hatching and other indices of reproductive toxicity.

3.1.10. Carcinogenicity and Mutagenicity

Trutter (1992a,b,c) assayed the potential carcinogenicity of tebufenozide in an 18-month bioassay in mice and a 24-month bioassay in rats. Both studies, summarized in Appendix 2, were accepted by the U.S. EPA (1999b). Moreover, neither of the two studies shows evidence of carcinogenicity.

Tebufenozide was assayed also for mutagenic activity in a number of test systems with uniformly negative results. At a maximum concentration of 5000 µg a.i./ plate, tebufenozide was not mutagenic to *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 with or without metabolic activation (S-9 liver fraction from Aroclor 1254 induced rats) (Black 1992; Sames and Elia 1993). In addition, tebufenozide did not induce gene mutations (HGPRT locus) in Chinese hamster ovary (CHO) cells with or without S-9 activation (Thilagar 1988, 1990a) and was also negative in an *in vivo* chromosome aberration assay in rat bone marrow cells (Gudi 1992). Finally, tebufenozide failed to induce DNA damage in primary rat hepatocytes (Thilagar 1990b).

Based on the lack of carcinogenic activity from *in vivo* assays and the lack of mutagenic activity in several *in vitro* assays, tebufenozide is classified as a Group E chemical (i.e., no evidence of carcinogenicity for humans) (U.S. EPA 1999b).

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

Tebufenozide was tested for toxic effects after dermal exposure as well as irritant effects on the skin and eyes of rabbits (Appendix 3). Technical grade tebufenozide does not appear to be an eye irritant (Hazleton and Quinn 1995b); nevertheless, a commercial formulation was shown to cause moderate eye irritation in rabbits (Gingrich and Parno 1994). The available studies on Mimic suggest that the other components in the formulation can cause skin irritation in rats (Morrison et al. 1993) and rabbits (Parno 1997). Neither tebufenozide nor Mimic, however, appear to cause skin sensitization in guinea pigs (Anderson and Shuey 1994; Glaza 1993).

The product label for Mimic advises that the formulation may cause moderate eye irritation and that contact with eyes, skin, or clothing should be avoided. This kind of advisory is, of course, standard and prudent practice for any chemical.

3.1.12. Systemic Toxic Effects from Dermal Exposure

Single dermal applications of technical grade tebufenozide are not toxic to rats at applied doses of up to 5000 mg/kg . These findings are consistent with the data indicating that tebufenozide has a low order of oral toxicity. Similarly, technical grade tebufenozide caused no signs of toxicity in rats and no hematological changes in rats when a dose of 1000 mg/kg was applied directly to the skin 5 days per week for 4 weeks (Hazleton and Quinn 1995b).

As indicated in Appendix 3, technical grade tebufenozide caused no signs of toxicity in rats and no change in hematological parameters in rats when applied directly to the skin at a dose of 1000 mg/kg, 6 hours per day, 5 days per week for 4 weeks (Hazleton and Quinn 1995b). Given the estimated first-order dermal absorption rate coefficient of 0.00317 $hour^{-1}$ (Section 3.1.3.2), the absorbed dose from this exposure may be estimated at about 13.5 mg/kg/day:

$$1000 \text{ mg/kg/day} \times (1-e^{-0.00317\times6}) \times 5/7 = 13.45 \text{ mg/kg/day}.$$

As also summarized by Hazleton and Quinn (1995b) and detailed in Appendix 2, dietary

concentrations of 1000 ppm tebufenozide for 2 weeks caused hematological effects in rats; however, the effects were not observed in rats exposed to 250 ppm. In this study, rats consumed food amounts equivalent to about 7% of their body weight per day. Thus, the dietary concentrations correspond to doses of 17.5 mg/kg/day (NOAEL of 250 ppm × 0.07 mg/kg per ppm) and 70 mg/kg/day (LOAEL of 1000 ppm × 0.07 mg/kg per ppm). Therefore, the estimate of the first-order dermal absorption rate is at least consistent with the comparable NOAEL values for oral and dermal exposures.

3.1.13. Inhalation Exposure

Acute inhalation studies are required for the registration of pesticides and three studies were submitted to U.S. EPA, one on technical grade tebufenozide, summarized by Hazleton and Quinn (1995b) and two conducted on wettable powder and LV Mimic formulations (Bemacki and Ferguson 1994a,b). At the highest technically achievable concentration of 0.43 mg/L, no mortality was observed in rats over a 2-week observation period after a single 4-hour exposure. At a concentration of 1.83 mg/L for 4 hours, the wettable formulation also caused no mortalities and no gross lesions (Bemacki and Ferguson 1994a). The liquid LV formulation, however, caused irritant changes in the respiratory tract after a single 4-hour exposure to 1.33 mg/L. Thus, as with dermal irritation, the liquid formulation of Mimic appears to be a greater irritant than tebufenozide.

These limited data suggest that the liquid formulation, LV Mimic, can induce irritant effects at very high exposure levels. Since the wettable powder did not produce irritant effects, the observed effects after exposure to LV Mimic may have been due to the presence of different materials in the LV Mimic formulation or due to the differences in the physical form – i.e., liquid and solid. As discussed in section 3.3, this effect by LV Mimic is not directly relevant to this risk assessment because of the implausibility of exposure to high concentrations of the compound.

3.1.14. Inerts and Adjuvants

Mimic contains materials other than technical grade tebufenozide that are included as inerts or adjuvants to improve either efficacy or ease of handling and storage. The identity of these materials is confidential. The additives were disclosed to the U.S. EPA and were reviewed in the preparation of this risk assessment. All that can be disclosed explicitly is that none of the additives is classified by the U.S. EPA as toxic.

Notwithstanding this assertion, it is apparent from a comparison of the acute dermal and inhalation data on technical grade tebufenozide and Mimic (see Sections 3.1.12 and 3.1.13) that Mimic contains materials that cause irritant effects not characteristic of technical grade tebufenozide. Thus, in terms of acute irritant effects that might be associated with the handling or application of Mimic, it is likely that the adjuvants or other inerts are of greater concern than tebufenozide. In terms of potential systemic toxic effects, however, there is no information to suggest that the adjuvants or inerts have an impact on the toxicity of this product.

3.1.15. Impurities and Metabolites

3.1.15.1. Impurities **–** There is no published information regarding the impurities in technical grade tebufenozide or any of its commercial formulations. Information on all of the impurities in technical grade tebufenozide were disclosed to the U.S. EPA, and the information was obtained and reviewed as part of this risk assessment (Kelly 1992). Because this information is classified as confidential business information, details about the impurities cannot be disclosed. Nonetheless, all of the toxicology studies on tebufenozide involve technical tebufenozide, which is presumed to be the same as or comparable to the active ingredient in the formulation used by the Forest Service. Thus, if toxic impurities are present in technical tebufenozide, they are likely to be encompassed by the available toxicity studies using technical grade tebufenozide.

3.1.15.2. Metabolites **–** As reviewed by the U.S. EPA (1999b), tebufenozide is subject to metabolism in mammals and more than 10 metabolites have been identified. The metabolic pathway appears primarily to involve oxidation of aliphatic groups on the benzyl rings to alcohols, aldehydes, or acids. No cleavage of the aliphatic rings has been noted. Since all of the *in vivo* toxicology studies on tebufenozide involve the generation of metabolites, the potential toxicity of the metabolites should be encompassed by the available toxicity data on tebufenozide. Major metabolites of tebufenozide have a low order of acute oral toxicity (LD_{50} values >5000 mg/k) and are inactive in bacterial mutagenicity assays (Quinn 1997).

3.1.16. Toxicologic Interactions

No information has been encountered on the toxicologic interactions of tebufenozide with other agents. As discussed in Section 3.1.2, tebufenozide causes methemoglobinemia in mammals. Many other chemicals may cause this effect and, as discussed in Section 3.4.5, interactions between tebufenozide and these agents are most likely to be additive rather than synergistic or antagonistic.

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview.

Standard sets of exposure scenarios are presented for both workers and members of the general public. The exposure assessments for these groups are summarized in Worksheet E01 (workers) and Worksheet E03 (general public). All exposure assessments are conducted at the maximum application rate for tebufenozide of 0.12 lb/acre using two applications with a minimum application interval of three days. This cumulative application (0.24 lb a.i./acre) is the maximum application rate for a single season. This leads to the highest estimates of peak as well as longer term exposures. The consequences of using lower application rates are discussed in the risk characterization (Section 3.4).

For workers applying tebufenozide, three types of application methods are modeled: directed ground spray, broadcast ground spray, and aerial spray. Central estimates of exposure for workers are approximately 0.002 mg/kg/day for aerial and backpack workers and about 0.003 mg/kg/day for broadcast ground spray workers. Upper ranges of exposures are approximately 0.02 mg/kg/day for broadcast ground spray workers and 0.01 mg/kg/day for backpack and aerial workers. All of the accidental exposure scenarios for workers involve dermal exposures and most of these accidental exposures lead to estimates of dose that are either in the range of or substantially below the general exposure estimates for workers. The one exception involves wearing contaminated gloves for one-hour. The upper range of exposure for this scenario is about 4 mg/kg/day.

For the general public, the range of acute exposures is from approximately 0.0000002 mg/kg associated with the lower range for the consumption of contaminated water from a stream by a child to 1.2 mg/kg associated with the upper range for consumption of contaminated water by a child after an accidental spill. Relatively high dose estimates are also associated with the direct spray of a child (about 0.4 mg/kg at the upper range of exposure) and for the consumption of fish after an accidental spill by members of the general public (0.2 mg/kg) and subsistence populations (0.9 mg/kg). Other acute exposure scenarios are associated with doses that are lower by at least an order of magnitude. For chronic or longer term exposures, the modeled exposures are much lower than for acute exposures, ranging from approximately 0.000000002 mg/kg/day (2 in 1 billionth of a mg/kg/day) associated with the lower range for the consumption of contaminated water to approximately 0.03 mg/kg/day associated with the upper range for consumption of contaminated fruit.

3.2.2. Workers.

The Forest Service uses a standard set of exposure assessments in all risk assessment documents. While these exposure assessments vary depending on the characteristics of the specific chemical as well as the relevant data on the specific chemical, the organization and assumptions used in the exposure assessments are standard and consistent. All of the exposure assessments for workers as well as members of the general public are detailed in the worksheets on tebufenozide that accompany this risk assessment (Supplement 1) and documentation for these worksheets is given in SERA (2003). A copy of this documentation is available at www.sera-inc.com. This

section on workers and the following section on the general public provides are plain verbal description of the worksheets and discuss tebufenozide specific data that are used in the worksheets.

A summary of the exposure assessments for workers is presented in Worksheet E01 of the worksheets for tebufenozide that accompany this risk assessment. Two types of exposure assessments are considered: general and accidental/incidental. The term *general* exposure assessment is used to designate those exposures that involve estimates of absorbed dose based on the handling of a specified amount of a chemical during specific types of applications. The accidental/incidental exposure scenarios involve specific types of events that could occur during any type of application. The exposure assessments developed in this section as well as other similar assessments for the general public (Section 3.2.3) are based on two applications spaced three days apart at the maximum single application rate of 0.12 lb/acre (Section 2). The consequences of using lower application rates are discussed further in the risk characterization (Section 3.4).

3.2.2.1. General Exposures – No studies on worker exposures to tebufenozide are available. As described in SERA (2001), worker exposure rates are expressed in units of mg of absorbed dose per kilogram of body weight per pound of chemical handled. Based on analyses of several different pesticides using a variety of application methods, default exposure rates are estimated for three different types of applications: directed foliar (backpack), boom spray (hydraulic ground spray), and aerial.

The specific assumptions used for each application method are detailed in Worksheets C01a (directed foliar), C01b (broadcast foliar), and C01c (aerial). In the worksheets, the central estimate of the amount handled per day is calculated as the product of the central estimates of the acres treated per day and the application rate.

Estimates of worker exposure rates are expressed in units of mg of absorbed dose per kilogram of body weight per pound of chemical handled. These estimates of exposure rates are based on worker exposure studies on nine different pesticides with molecular weights ranging from 221 to 416 and log K_{ow} values ranging from -0.75 to 6.50. The estimated exposure rates are based on estimated absorbed doses in workers as well as the amounts of the chemical handled by the workers. As summarized in Table 2-1 of this risk assessment, the molecular weight of tebufenozide is 352.48 and the log K_{ow} is about 4.25. These values are within the range of the pesticides used in SERA (2001) to estimate worker exposures. As discussed in SERA (2001), the ranges of estimated occupational exposure rates vary substantially among individuals and groups, (i.e., by a factor of 50 for backpack applicators and a factor of 100 for mechanical ground sprayers). It seems that much of the variability can be attributed to the hygienic measures taken by individual workers (i.e., how careful the workers are to avoid unnecessary exposure); however, pharmacokinetic differences among individuals (i.e., how individuals absorb and excrete the compound) also may be important.

The number of acres treated per hour is taken from previous USDA risk assessments (USDA 1989a,b,c). The number of hours worked per day is expressed as a range, the lower end of which is based on an 8-hour work day with 1 hour at each end of the work day spent in activities that do not involve exposure to the compound. The upper end of the range, 8 hours per day, is based on an extended (10-hour) work day, allowing for 1 hour at each end of the work day to be spent in activities that do not involve exposure to the chemical.

It is recognized that the use of 6 hours as the lower range of time spent per day applying herbicides is not a true lower limit. It is conceivable and perhaps common for workers to spend much less time in the actual application of a herbicide if they are engaged in other activities. Thus, using 6 hours may overestimate exposure. In the absence of any published or otherwise documented work practice statistics to support the use of a lower limit, this approach is used as a protective assumption.

The range of acres treated per hour and hours worked per day is used to calculate a range for the number of acres treated per day. For this calculation as well as others in this section involving the multiplication of ranges, the lower end of the resulting range is the product of the lower end of one range and the lower end of the other range. Similarly, the upper end of the resulting range is the product of the upper end of one range and the upper end of the other range. This approach is taken to encompass as broadly as possible the range of potential exposures.

The central estimate of the acres treated per day is taken as the arithmetic average of the range. Because of the relatively narrow limits of the ranges for backpack and boom spray workers, the use of the arithmetic mean rather than some other measure of central tendency, like the geometric mean, has no marked effect on the risk assessment.

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

Tebufenozide may cause eye irritation (Section 3.1.11). The available literature does not include quantitative methods for characterizing exposure or responses associated with splashing a solution of a chemical into the eyes; furthermore, there appear to be no reasonable approaches to modeling this type of exposure scenario quantitatively. Consequently, accidental exposure scenarios of this type are considered qualitatively in the risk characterization (section 3.4).

As detailed in Section 3.1.3, there are various methods for estimating absorbed doses associated with accidental dermal exposure (U.S. EPA 1992; SERA 2001). Two general types of exposure are modeled: those involving direct contact with a solution of the herbicide and those associated with accidental spills of the herbicide onto the surface of the skin. Any number of specific

exposure scenarios could be developed for direct contact or accidental spills by varying the amount or concentration of the chemical on or in contact with the surface of the skin and by varying the surface area of the skin that is contaminated.

For this risk assessment, 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 summarize in Worksheet E01, which references other worksheets in which the specific calculations are detailed.

Exposure scenarios involving direct contact with solutions of the chemical are characterized by immersion of the hands for 1 minute or wearing contaminated gloves for 1 hour. Generally, it is not reasonable to assume or postulate that the hands or any other part of a worker will be immersed in a solution of a herbicide for any period of time. On the other hand, contamination of gloves or other clothing is quite plausible. For these exposure scenarios, the key element is the assumption that wearing gloves grossly contaminated with a chemical solution is equivalent to immersing the hands in a solution. In either case, the concentration of the chemical in solution that is in contact with the surface of the skin and the resulting dermal absorption rate are essentially constant.

For both scenarios (the hand immersion and the contaminated glove), the assumption of zero-order absorption kinetics is appropriate. Following the general recommendations of U.S. EPA/ORD (1992), Fick's first law is used to estimate dermal exposure. As discussed in Section 3.1.3, an experimental dermal permeability coefficient (Kp) for tebufenozide is not available. Thus, the Kp for tebufenozide is estimated using the algorithm from U.S. EPA (1992a).

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

For both scenarios, it is assumed that the contaminated skin is effectively cleaned after 1 hour. As with the exposure assessments based on Fick's first law, this product (mg of absorbed dose) is divided by body weight (kg) to yield an estimated dose in units of mg chemical/kg body weight.

3.2.3. General Public.

3.2.3.1. General Considerations – Although some applications of tebufenozide may be made in relatively remote areas involving limited exposure to the general public, both aerial and ground applications may be made in residential areas. In residential applications, members of the general public are likely to be exposed to tebufenozide. Any number of exposure scenarios can be constructed for the general public, depending on various assumptions regarding application rates, dispersion, canopy interception, and human activity. Several scenarios are developed for this risk assessment which should tend to over-estimate exposures in general.

The two types of exposure scenarios developed for the general public include acute exposure and longer-term or chronic exposure. All of the acute exposure scenarios are primarily accidental. They assume that an individual is exposed to the compound either during or shortly after its application. Specific scenarios are developed for direct spray, dermal contact with contaminated vegetation, as well as the consumption of contaminated fruit, water, and fish. Most of these scenarios should be regarded as extreme, some to the point of limited plausibility. The longer-term or chronic exposure scenarios parallel the acute exposure scenarios for the consumption of contaminated fruit, water, and fish but are based on estimated levels of exposure for longer periods after application.

The exposure scenarios developed for the general public are summarized in Worksheet E03. As with the worker exposure scenarios, details of the assumptions and calculations involved in these exposure assessments are given in the worksheets that accompany this risk assessment (Worksheets D01a to D09b). The remainder of this section focuses on a qualitative description of the rationale for and quality of the data supporting each of the assessments.

3.2.3.2. Direct Spray – Direct sprays involving ground applications are modeled in a manner similar to accidental spills for workers (Section 3.2.2.2). In other words, it is assumed that the individual is sprayed with a solution containing the compound and that an amount of the compound remains on the skin and is absorbed by first-order kinetics. For these exposure scenarios, it is assumed that during a ground application, a naked child is sprayed directly with tebufenozide. These scenarios also assume that the child is completely covered with tebufenozide (that is, 100% of the surface area of the body is exposed and contaminated). These exposure scenarios are likely to represent upper limits of plausible exposure. An additional set of scenarios are included involving a young woman who is accidentally sprayed over the feet and legs. For each of these scenarios, some assumptions are made regarding the surface area of the skin and body weight. These are detailed in Worksheets B05, B06, and B07, for an adult male, and adult female, and a young child, respectively.

3.2.3.3. Dermal Exposure from Contaminated Vegetation – In this exposure scenario, it is assumed that the herbicide is sprayed at a given application rate and that an individual comes in contact with sprayed vegetation or other contaminated surfaces at some period after the spray operation. For these exposure scenarios, some estimates of dislodgeable residue and the rate of transfer from the contaminated vegetation to the surface of the skin must be available. No such

data are available on dermal transfer rates for tebufenozide and the estimation methods of Durkin et al. (1995) are used as defined in Worksheet D02. The exposure scenario assumes a contact period of one hour and assumes that the chemical is not effectively removed by washing until 24 hours after exposure. Other estimates used in this exposure scenario involve estimates of body weight, skin surface area, and first-order dermal absorption rates, as discussed in the previous section.

3.2.3.4. Contaminated Water – Water can be contaminated from runoff, as a result of leaching from contaminated soil, from a direct spill, or from unintentional contamination from aerial applications. For this risk assessment, three exposure scenarios are considered for the acute consumption of contaminated water: an accidental spill into a small pond (0.25 acres in surface area and 1 meter deep), accidental direct spray of or incidental drift into a pond and stream, and the contamination of a small stream and pond by runoff or percolation. In addition, longer-term estimates of concentrations in water are based on a combination of modeling and monitoring data. Each of these scenarios are considered in the following subsections.

3.2.3.4.1. Accidental Spill – The accidental spill scenario assumes that a young child consumes contaminated water shortly after an accidental spill into a small pond. The specifics of this scenarios are given in Worksheet D05. Because this scenario is based on the assumption that exposure occurs shortly after the spill, no dissipation or degradation of tebufenozide is considered. This scenario is dominated by arbitrary variability and the specific assumptions used will generally overestimate exposure. The actual concentrations in the water would depend heavily on the amount of compound spilled, the size of the water body into which it is spilled, the time at which water consumption occurs relative to the time of the spill, and the amount of contaminated water that is consumed. Based on the spill scenario used in this risk assessment, the concentration of tebufenozide in a small pond is estimated to range from about 0.22 mg/L to 11 mg/L with a central estimate of about 2.2 mg/L (Worksheet D05). This is and is intended to be an extreme accidental exposure scenario. The purpose of this scenario is simply to suggest the intensity of measures that would need to be taken in the event of a relatively large spill of tebufenozide into a relatively small body of water.

3.2.3.4.2. Accidental Direct Spray/drift for a Pond or Stream – These scenarios are less severe but more plausible than the accidental spill scenario described above. The U.S. EPA typically uses a two meter deep pond to develop exposure assessments (SERA 2004b). If such a pond is directly sprayed with tebufenozide at the nominal application rate of 0.12 lb/acre, the peak concentration in the pond would be about 0.0067 mg/L, equivalent to 6.7 µg/L or 6.7 ppb (Worksheet D10a). This concentration is a factor of about 325 below central estimate of the peak concentration of 2.2 mg/L after the accidental spill (Worksheet D05). Because the USDA will not directly spray open bodies of water, the concentration of 0.0067 mg/L from direct spray would be an accidental exposure. At distances of 100 to 500 feet down wind, estimates of drift of tebufenozide from aerial applications would result in water concentrations between about 0.000015 mg/L (500 feet) to about 0.00013 mg/L (100 feet) (Worksheet D10a).

Similar calculations can be made for the direct spray of a stream and the resulting water concentrations will be dependant on the surface area of the stream that is sprayed and the rate of water flow in the stream. The stream modeled using GLEAMS (see below) is about 6 feet wide (1.82 meters) and it is assumed that the pesticide is applied along a 1038 foot (316.38 meters) length of the stream with a flow rate of 710,000 L/day. An application rate of 0.12 lb/acre, is equivalent to 13.45 mg/m^2 [0.12 lb/acre × 112.1 mg/m^2 per lb/acre]. Thus, a direct spray would be equivalent to about 7745 mg [1.82 meters × 316.38 meters × 13.45 mg/m^2]. The daily average concentration in the stream segment would be about 0.011 mg/L [7745 mg ÷ 710,000 L/day]. Instantaneous concentrations would, of course, vary remarkably over time during and after drift. If the stream were 100 feet downwind of the application site, the drift would be a factor of 0.0195 of the application rate (Worksheet B23). Thus, the average daily concentration in the stream would be about 0.2 µg/L [0.011 mg/L × 0.0195 = 0.00021 mg/L or 0.21 µg/L]. Similar calculations for other distances are summarized in Worksheet D10b.

3.2.3.4.3. Gleams Modeling – For compounds such as tebufenozide, which may be applied over a large proportion of a watershed, drift and even direct spray are not the only and may not be the greatest source of contamination of surface water. Water contamination may also occur from soil runoff or percolation and, depending on local conditions, can lead to substantial contamination of ponds or streams. Estimates of these concentrations can be based both on modeling and monitoring data.

Modeling of concentrations in stream water conducted for this risk assessment are based on GLEAMS (Groundwater Loading Effects of Agricultural Management Systems) modeling. GLEAMS is a root zone model that can be used to examine the fate of chemicals in various types of soils under different meteorological and hydrogeological conditions (Knisel and Davis 2000). As with many environmental fate and transport models, the input and output files for GLEAMS can be complex. The general application of the GLEAMS model and the use of the output from this model to estimate concentrations in ambient water are detailed in SERA (2004b).

For the current risk assessment, the application site was assumed to consist of a 10 hectare square area that drained directly into a small pond or stream. The chemical specific values as well as the details of the pond and stream scenarios used in the GLEAMS modeling are summarized in Table 3-1. The GLEAMS modeling yielded estimates of runoff, sediment and percolation that were used to calculate concentrations in the stream adjacent to a treated plot, as detailed in Section 6.4 of SERA (2004b). The results of the GLEAMS modeling for the small stream are summarized in Table 3-2 and the corresponding values for the small pond are summarized in Table 3-3. These estimates are expressed as both average and maximum concentrations in water. The top section of each table gives the water contamination rates (WCR) – i.e., the concentration of the compound in water in units of ppb (µg/L) normalized for an application rate of 1 lb/acre. The bottom section of each table gives the estimated maximum and average concentrations adjusted for the two applications spaced three days apart at a rate of 0.12 lb/acre (Section 2.3).

At the application rate of 0.12 lb/acre, no stream contamination is estimated in very arid regions

– i.e., annual rainfall of 10 inches of less. At higher rainfall rates, the modeled peak concentrations in streams range from about 0.04 µg/L (loam at an annual rainfall rate of 15 inches) to about 40 µg/L (clay soil at an annual rainfall rate of 150 inches per year) (Table 3-2). While not detailed in Table 3-2, the losses from clay are about equally divided between sediment loss (about 51%) and runoff loss (about 49%). Water contamination due to percolation is negligible (a proportion of about 8×10^{-9}). In sandy soils, however, percolation accounts for virtually all of the total loss at an annual rainfall rate of 250 inches.

Modeled concentrations in a small pond (Table 3-3) are lower than those modeled in the stream. As with the stream modeling, no surface water contamination is expected in very arid regions. For regions with annual rainfall rates of 15 inches or more, the modeled peak concentrations in ponds range from less than 0.006 µg/L (loam) to about 20 µg/L (clay soil at an annual rainfall rate of 250 inches per year). The GLEAMS scenarios do not specifically consider the effects of accidental direct spray. As discussed above and detailed in Worksheet A04b, direct spray of a standard pond could result in peak concentrations of about 6.7 µg/L, somewhat less than the 20 µg/L peak concentration modeled in ponds.

3.2.3.4.4. Other Modeling Efforts – A summary of the GLEAMS modeling discussed above as well as modeling of tebufenozide conducted for other analyses is given in Table 3-4. In addition to GLEAMS, two other water contamination models were used: GENEEC and Sci-Grow. As discussed in SERA (2004b), these are Tier 1 screening models developed by the U.S. EPA that are intended to provide very conservative upper range estimates of concentrations of a compound in surface water (GENEEC) and groundwater (Sci-Grow) based on a given application rate, number of applications, the interval between applications, and standard environmental fate parameters for a specific compound (i.e., a subset of those summarized in Table 3-1).

Estimates of peak concentrations from GENEEC, about 8µg/L, are similar to the central estimates from GLEAMS, 5 to 10 µg/L, but are somewhat less than the peak estimates from GLEAMS, 20 to 40 µg/L. This suggests that although GENEEC is designed as a very conservative model, the application of GLEAMS to the modeling for tebufenozide incorporated more extreme scenarios for contamination. As detailed in SERA (2004b), the application of GLEAMS is intended to encompass extreme situations which favor high runoff from clay and high percolation losses from sand. GENEEC does not provide direct estimates of annual average concentration but does provide 90-day average concentrations. Adjusting the GENEEC modeled 90-day average of 6 µg/L over a one-year period, the concentration of 1.5 µg/L is very close to the upper range of the average concentration modeled using GLEAMS – i.e., 1.4 µg/L for the pond. Sci-Grow estimates a ground water concentration of about 0.09 µg/L. This is in the lower range of the estimates from GLEAMS. This is probably due to the very shallow root zone used in the GLEAMS modeling – i.e., 12 inches – compared to the 8 to 25 feet water table depth used in Sci-Grow (http://www.epa.gov/oppefed1/models/water/scigrow_description.htm#characteristics).

The only other modeling effort encountered for tebufenozide is the use of PRZM/EXAMS by the U.S. EPA (1999e) for the reregistration of tebufenozide. As summarized in Table 3-4, the U.S. EPA (1999e) modeled the application of tebufenozide to an apple orchard (6 applications at 0.31 lb/acre) and to a cotton field (4 applications at 0.25 lb/acre) for a pond. While this modeling effort used assumptions and weather data substantially different from the GLEAMS modeling (i.e., application rates, soil types, and rainfall patterns), the results are reasonably consistent with the above estimates of concentrations in surface waters based on GLEAMS correcting for differences in the total amount of tebufenozide applied. In the modeling of applications to cotton at a cumulative application rate of 1 lb/acre, for example, the peak concentration estimated by U.S. EPA (1999e) is 17 µg/L. The GLEAMS model was run at a cumulative application of 0.24 lb/acre and the adjusted peak concentration for a pond from U.S. EPA (1999e) would be about 4 µg/L [17 µg/L × 0.24 = 4.08 µg/L], very close to the central estimate of 5 µg/L modeled using GLEAMS. The average annual concentration modeled by U.S. EPA (1999e) was about 8.2 µg/L, which would correspond to 2 µg/L [8.2 µg/L × 0.24 = 1.96 µg/L] at an application rate of 0.24 lb/acre. This is only modestly higher than the peak concentration from GLEAMS of 1.4 µg/L.

3.2.3.4.5. Monitoring Data – Very little water monitoring data are available on tebufenozide. Although the USGS (1998) provides information on the agricultural uses of tebufenozide, no monitoring data on tebufenozide are available from the USGS National Water Quality Assessment (NAWQA). Sundaram et al. (1996a) published a monitoring study of concentrations of tebufenozide in water that might be associated with the application of this pesticide in a forest environment. In this study, tebufenozide was aerially applied at a rate of 70 g/ha (0.07 kg/ha or 0.06244 lb/acre) to a 500 ha boreal forest. Two applications were made at 4 days apart. Water concentrations were then monitored in a small pond and stream. The pond had a surface area of 500 m^2 and an average depth of 0.6 m for a volume of 300 m^3 or 300,000 L [1,000 L/m^3]. Water concentrations were monitored at 1 , 8, and 12 hours after application as well as 1, 2, 3, 4, 5, 8, 12, and 24 days after application.

The peak concentration, 5.31 ppb (0.00531 mg/L) occurred 1 hour after the first application, clearly indicating that the water had been directly sprayed. Taking the water volume of 300,000 L, the amount applied to the pond can be calculated as, 1,593 mg,

$$0.00531 \text{ mg/L} \times 300{,}000 \text{ L}.$$

The nominal application rate of 0.07 kg/ha is equivalent to 70,000 mg/10,000 m^2 or 7 mg/m^2. At this nominal application rate, the total amount applied to a 500 m^2 pond would be 3500 mg,

$$7 \text{ mg/m}^2 \times 500 \text{ m}^2.$$

Thus, it appears that the initial concentrations of tebufenozide in water are consistent with the direct spray of about 50% [1,593 mg/3500 mg = 0.455 ≈50%] of the pond at the nominal application rate.

3.2.3.4.6. Concentrations of Tebufenozide in Water Used for Risk Assessment – A summary of the concentrations of tebufenozide in water that are used for the current risk assessment is given in Table 3-5. The upper range of the expected peak concentration of tebufenozide in surface water will be taken as 40 µg/L. This is based on the upper range of concentrations estimated in streams from the GLEAMS modeling. This concentration also encompasses accidental direct sprays of both a small stream and small pond (Table 3-4). In most instances, concentrations in surface water are likely to be much lower. At the lower extreme, an argument may be made that concentrations of tebufenozide are likely to be essentially zero – i.e., applications at sites that are distant from open bodies of water and in areas in which runoff or percolation are not likely to occur. For this risk assessment, the lower range of the peak concentration in ambient water will be set at 0.005 µg/L. This is in the lower range of non-zero concentrations modeled in streams and ponds in relatively arid regions. The central estimate of concentration of tebufenozide in surface water will be taken as 10 µg/L. This is the central estimate of the concentrations modeled in ponds (Table 3-4).

Longer term concentrations of tebufenozide in surface water will be much lower than peak concentrations. At an application rate of 0.12 lb/acre, the highest longer term concentration will be taken as 1.4 µg/L. This is the maximum longer term concentration modeled using GLEAMS and is near the maximum longer term concentration given by U.S. EPA (1999e) after adjusting for differences in application rate. As with peak concentrations, the lower range of longer term concentrations will approach zero. For this risk assessment, the lower range of longer term concentrations is taken as 0.002 µg/L, the lowest non-zero value modeled for tebufenozide in ponds at the application rate of 0.12 lb/acre. This lower range is somewhat arbitrary but has no impact on the risk assessment. The central value for longer term concentrations of tebufenozide in water will be taken as 0.5 µg/L. This is the central estimate of the longer term concentrations in ponds modeled using GLEAMS and is somewhat higher than the central estimate of the longer term concentration in streams (Table 3-4).

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

The bioconcentration of tebufenozide was determined in fathead minnows (Rhodes and Leak 1996) and bluegill sunfish (Dong and Hawkins, 1993). In fathead minnows, bioconcentration factors (BCF) range from about 17 in pre-spawn adults to greater than 100 in newly fertilized embryos (Rhodes and Leak 1996). In bluegills, Dong and Hawkins (1993) provide data on bioconcentration in the edible muscle (BCF=7.5) as well as viscera (BCF=106) and whole body

(BCF=52). For the human health risk assessment, the bioconcentration factor of 7.5 from Dong and Hawkins (1993) is used. Taking the value for the edible portion of fish is not the most conservative approach but seems the most realistic approach because humans usually clean caught fish and consume only the fillet or muscle. For the ecological risk assessment, however, the higher BCF value of 52 (whole body) is used.

For the acute and longer-term exposure scenarios involving the consumption of contaminated fish, the water concentrations of tebufenozide used are identical to the concentrations used in the contaminated water scenarios (Section 3.2.3.4.6). The acute exposure scenario is based on the assumption that an adult angler consumes fish taken from contaminated water shortly after an accidental spill of 200 gallons of a field solution into a pond that has an average depth of 1 m and a surface area of 1000 m^2 or about one-quarter acre. No dissipation or degradation is considered.

Bioconcentration is a dynamic process and for some compounds time to maximum steady state may be prolonged. For tebufenozide, Dong and Hawkins (1993) found that time to steady state was reached in about 1-day. Thus, the use of the experimental BCF for the acute accidental scenario is not overly conservative. Nonetheless, this scenario may somewhat overestimate exposure in that some degradation of tebufenozide could occur during the course of the acute spill scenario.

Because of the available and well-documented information and substantial differences in the amount of caught fish consumed by the general public and native American subsistence populations (U.S. EPA 1996), separate exposure estimates are made for these two groups, as illustrated in Worksheet D08a and D08b. The chronic exposure scenario is constructed in a similar way, as detailed in Worksheets D09a and D09b.

3.2.3.6. Oral Exposure from Contaminated Vegetation – Although Forest Service applications of tebufenozide will not involve the intentional treatment of food crops, incidental exposure to vegetation that may be consumed by members of the general public is plausible during broadcast applications. Any number of scenarios could be developed involving either accidental spraying of crops or the spraying of edible wild vegetation, like berries. The exposure scenarios developed for this exposure assessment include one scenario for acute exposure, as defined in Worksheet D03 and two scenarios for longer-term exposure, as defined in Worksheets D04a and D04b. In both acute and longer-term scenarios, the concentration of tebufenozide on contaminated vegetation is estimated using the empirical relationships between application rate and concentration on vegetation developed by Fletcher et al. (1994) which is in turn based on a re-analysis of data from Hoerger and Kenaga (1972). These relationships are defined in Worksheet B20.

For the acute exposure scenario involving only a single application (Worksheet D03a), the estimated residue level is taken as the product of the application rate and the residue rate for contaminated fruit. 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 (C_0), the number of applications (n), and the first-order decay coefficient (k), which can be calculated from the halftime (t_{50}) [$k=\ln(2)\div t_{50}$]. Assuming a first-order decrease in concentrations in contaminated vegetation, the concentration in the vegetation at time t after the first application (C_t), can be calculated as:

$$C_t = C_0 \times e^{-kt} \qquad \text{(Eq. 3-1)}$$

Using the plateau principle (e.g., Goldstein et al. 1974, p. 321) and defining Δt as the interval between applications and $e^{-k\,\Delta t}$ as p to simplify notation, the concentration immediately after the n^{th} application (C_n) can be calculated as:

$$C_n = C_0 \times (1 - p^n) \div (1 - p). \qquad \text{(Eq. 3-2)}$$

This algorithm is used in Worksheet D03b to calculate the maximum concentration on vegetation after multiple applications at the specified interval.

For the longer-term exposure scenario (Worksheets D04a and D04b), a duration of 90 days is used. Although the duration of exposure of 90 days is somewhat arbitrarily, 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 would lower the estimated dose (i.e., would reduce the estimate of risk).

The reported halftimes on vegetation are highly variable (Table 2-1), ranging from 2.8 days, the lower value of the range reported by Hawkins (1998) to 58.7 days, the upper value of the range reported by Sundaram et al. (1996a). This 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 the type of vegetation. For this risk assessment, the range of vegetation halftimes will be taken as 3 to 60 days (the approximate range summarized in Table 2-1) and the central estimate will be taken as 13.4 days, the geometric mean of this range.

For the longer-term exposure scenarios, the time-weighted average concentration on fruit is calculated from the equation for first-order dissipation. Assuming a first-order decrease in concentrations in contaminated vegetation, the concentration in the vegetation at time t after spray, C_t, can be calculated based on the initial concentration, C_0, as:

$$C_t = C_0 \times e^{-kt}$$

where k is the first-order decay coefficient which can be calculated from the halftime (t_{50}) [$k=\ln(2)\div t_{50}$]. For a single application, the time-weighted average concentration (C_{TWA}) over time t can be calculated as the integral of C_t (De Sapio 1976, p. p. 97 ff) divided by the duration (t):

$$C_{TWA} = C_0 (1 - e^{-k\ t}) \div (k\ t).$$

This equation is used to estimate the time-weighted average concentration on vegetation after a single applications (Worksheet D04a).

For two applications, such as those modeled in this risk assessment, the expression of the time-weighted average concentration is somewhat more complicated. Defining *exp(x)* as e^x, where *x* is any number, the time-weighted average concentration over a period from the day of application to time t_2 with a second application occurring on day t_1 (where $t_1 \leq t_2$) is:

$$C_{TWA} = (C_0 (1-\exp(-kt_1)) + [\{C_0 + C_0 \exp(-kt_1)\} \times \{1-\exp(-k [t_2 - t_1])\}]) \div (k t_2)$$

This equation is used to estimate the time-weighted average concentration on vegetation after a single applications (Worksheet D04b).

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview

Acute and chronic risk values are derived for tebufenozide. Following standard practices for USDA risk assessments, risk assessment values available from U.S. EPA are adopted directly unless there is a compelling basis for doing otherwise. When risk values are not available from U.S. EPA, the methods used by U.S. EPA are employed to derive surrogate values.

U.S. EPA has derived a chronic RfD for tebufenozide of 0.018 mg/kg/day. This chronic RfD is well-documented and is used directly for all longer term exposures to tebufenozide. This value is based on a NOAEL of 1.8 mg/kg/day in dogs and an uncertainty factor of 100 – two factors of 10 for interspecies and intraspecies variability. Because of the low acute toxicity of tebufenozide, the U.S. EPA has not derived an acute RfD but has identified an acute NOAEL of 1000 mg/kg/day from reproduction studies in both rats and rabbits involving 10 to 13 day exposure periods. This NOAEL is the basis for a surrogate acute RfD of 10 mg/kg using an uncertainty factor of 100 as in the chronic RfD. This surrogate acute RfD is applied to all incidental or accidental exposures that involve an exposure period of 1 day.

3.3.2. Chronic RfD

The most recent RfD for tebufenozide is 0.018 mg/kg/day, a value derived by the U.S. EPA's Office of Pesticide Programs (U.S. EPA 1999b,e). This compound is not listed on the U.S. EPA's agency-wide list of approved RfDs (i.e., IRIS) (U.S. EPA 2004). As noted in section 3.1.2 and detailed in Appendix 2, the most sensitive endpoint for tebufenozide is hematological effects including methemoglobin formation and several other endpoints that are characteristic of hemolytic anemia. These effects were observed in mice, rats, and dogs, with the dog being the most sensitive species tested with tebufenozide. As reviewed by Calabrese (1991), this pattern is consistent with known differences in methemoglobin reductase activity which suggest that the cat may be the most sensitive species, followed by humans (half as susceptible as cats), dogs (half as susceptible as human), and rats (about one-tenth as susceptible as humans).

The RfD derived by the U.S. EPA (1999b) is based on a study by Richards (1992a,b) in which a dietary concentration of 0, 15, 50, 250, or 1500 ppm technical grade tebufenozide was provided to male and female beagles for 52 weeks (Appendix 2). In the 250 and 1500 ppm groups, the primary hematological effects were increased concentrations of methemoglobin. The increases in methemoglobin concentrations were associated with increased breakdown of red blood cells in the liver and spleen, and decreases in red blood cell counts, hemoglobin concentrations, and packed red cell volume, along with several other associated hematological effects. None of these effects were observed in beagles exposed to a dietary concentration of 50 ppm technical grade tebufenozide, which corresponded to a daily dose of 1.5-2.4 mg/kg bw (based on measured food consumption). Taking 1.8 mg/kg bw/day as a central estimate of the NOAEL, the U.S. EPA (1999b) applied an uncertainty factor of 100, two factors of 10 for interspecies and intraspecies variability, to arrive at the chronic RfD of 0.018 mg/kg/day.

Under the Food Quality Protection Act (FQPA), the U.S. EPA is required to consider an

additional uncertainty factor of 10 for the protection of infants and children. For tebufenozide, the U.S. EPA (1999b) determined that the additional uncertainty factor is not required because of the information indicating that tebufenozide does not have developmental or reproductive effects at doses below those associated with hematological effects. Hence, because the RfD should protect against hematological effects, it should also protect against developmental or reproductive effects. As discussed in Section 3.4.4, infants less than three months old have lower levels of methemoglobin reductase than older children or adults and may be more sensitive tebufenozide and other agents that cause methemoglobinemia. While it may be argued that an uncertainty factor for very young children might be appropriate, this would not have an impact on the risk characterization because of the very low hazard quotients associated with various exposure scenarios for tebufenozide (Section 3.4.3).

3.3.4. Acute RfD

The U.S. EPA (1999b) considers the acute and intermediate risk from acute or intermediate exposure to tebufenozide negligible and does not propose short-term or intermediate-term criteria for exposure to tebufenozide. Specifically, the U.S. EPA (1999b) made the following judgement:

> *1. Acute toxicity. Toxicity observed in oral toxicity studies were not attributable to a single dose (exposure). No neuro or systemic toxicity was observed in rats given a single oral administration of tebufenozide at 0, 500, 1,000, or 2,000 mg/kg. No maternal or developmental toxicity was observed following oral administration of tebufenozide at 1,000 mg/kg/day (Limit-Dose) during gestation to pregnant rats or rabbits. Thus, the risk from acute exposure is considered negligible.*
>
> *2. Short- and intermediate-term toxicity. No dermal or systemic toxicity was seen in rats receiving 15 repeated dermal applications of the technical (97.2%) product at 1,000 mg/kg/day (Limit-Dose) as well as a formulated (23% a.i.) product at 0, 62.5, 250, or 1,000 mg/kg/day over a 21-day period. The Agency noted that in spite of the hematological effects seen in the dog study, similar effects were not seen in the rats receiving the compound via the dermal route indicating poor dermal absorption. Also, no developmental endpoints of concern were evident due to the lack of developmental toxicity in either rat or rabbit studies. This risk is considered to be negligible.* -- U.S. EPA (1999b).

In paragraph 1 above, the acute toxicity study with a single-dose NOAEL of 2000 mg/kg appears to refer to the study by Swenson et al. (1994) and the NOAEL of 1000 mg/kg/day for maternal toxicity and reproductive effects in rats and rabbits appears to refer to the studies by Hoberman (1991) and Swenson and Solomon (1992), respectively. In paragraph 2 above, the U.S. EPA (1999b) refers to a dermal study with a NOAEL of 1000 mg/kg/day. In this study, tebufenozide

was applied 5 days per week for three weeks – i.e., 15 exposures over a 21 day period. Two repeated dermal dose studies have been identified with a NOAEL of 1000 mg/kg/day (Hazleton and Quinn 1995b; Morrison et al. 1993). As summarized in Appendix 3, both of these studies report exposure periods of 4 weeks rather than 3 weeks.

While the decision of the U.S. EPA (1999b) to classify acute and short-term risks associated with tebufenozide appears reasonable, the failure of the U.S. EPA (1999b) to derive an acute RfD limits the ability to quantitatively characterize risks associated with acute exposures. As detailed in Section 3.2, the current risk assessment is concerned with characterizing the risks of several acute exposure scenarios. In addition, the current risk assessment is part of a series of risk assessments on different agents used to control the gypsy moth the estimates of risks from the various agents will be compared in a companion document.

Consequently, this risk assessment will use a surrogate acute RfD. Typically, the U.S. EPA will base acute RfDs on reproduction studies, specifically teratology studies that involve multiple daily gavage doses to pregnant animals. For the current risk assessment, the NOAEL of 1000 mg/kg/day in pregnant rats and rabbits identified by U.S. EPA (1999b) will be used As detailed in Appendix 2, the NOAEL in rabbits is from a study (Swenson and Solomon 1992) in which animals were dosed on Days 7-19 of gestation – i.e., repeated exposures over 13 days – and the NOAEL in rats is from a study (Hoberman 1991) in which animals were dosed on Days 6-15 of gestation – i.e., repeated exposures over 10 days. Dividing this NOAEL by an uncertainty factor of 100, identical to that used by U.S. EPA (1999b) in the chronic RfD, yields a surrogate acute RfD of 10 mg/kg/day. This value is used to characterize risks associated to incidents or accidents that involve an exposure period of 1 day.

3.4. RISK CHARACTERIZATION

3.4.1. Overview

At the maximum application rate considered in this risk assessment, two applications at 0.12 lb/acre spaced three day apart, there is little indication that adverse effects on human health are likely. Based on central estimates of exposure – those that might be considered typical and expected – hazard quotients including workers and members of the general public range from 0.00003 to 0.03, below a level of concern by factors of about 30 to 33,000. At the upper range of plausible exposures, the hazard quotient for ground spray workers reaches a level of concern – i.e., a hazard quotient of 1. For members of the general public, the upper range of exposure leads to a hazard quotient of 1.5 for the longer-term consumption of contaminated vegetation for two applications at 0.12 lb/acre. Because of the linear relationship between exposure and application rate, two applications at 0.08 lb/acre would reach but not exceed a level of concern. With a single application at the maximum rate of 0.12 lb/acre, the hazard index is 0.8, below the level of concern. While the longer-term consumption of contaminated vegetation is probably not a likely scenario, it is a standard exposure scenario used in Forest Service risk assessments to consider the longer-term consumption of food items such as berries that might be sprayed during the broadcast application of a pesticide. This risk assessment suggests that two applications at 0.08 lb/acre or more should be avoided in areas where members of the general public might consume contaminated fruits or other contaminated vegetation.

3.4.2. Workers

A quantitative summary of the risk characterization for workers is presented in Worksheet E02 (Supplement 1). The quantitative risk characterization is expressed as the hazard quotient, which is the ratio of the estimated exposure from Worksheet E01 to the RfD. For acute accidental/incidental exposures, the surrogate acute RfD of 10 mg/kg is used (Section 3.3.3). For longer term general exposures – i.e., exposures that could occur over the course of several days, weeks, or months during an application season – the chronic RfD of 0.018 mg/kg/day is used (Section 3.3.2).

At the maximum application rate considered in this risk assessment, 0.12 lb/acre, none of the acute hazard quotients exceed a level of concern – i.e., a hazard quotient of 1. The highest acute hazard quotient is 0.4, associated with wearing contaminated gloves for 1 hour. It should be noted, however, that the magnitude of the hazard quotient is linearly related to the duration of exposure. The 1-hour exposure period is simply a convention that is uniformly used in Forest Service risk assessments (SERA 2001). For tebufenozide, the estimated exposure would exceed the acute RfD – i.e., result in a hazard quotient greater than 1 – if a worker were to wear contaminated gloves for a period greater than 2.5 hours. Thus, the exposure involving contaminated gloves is of greatest concern and this concern would apply to wearing any clothing that is saturated with tebufenozide.

For longer-term exposures, the highest hazard quotient is 1.008 and is associated with the upper range of exposure for ground spray workers at the maximum application rate of 0.12 lb/acre. In Worksheet E02, this value is presented as 1.0 – i.e., rounded to one significant place after the

decimal. This very minor exceedence of the chronic RfD is interpreted as a hazard quotient of 1.0 – i.e., the level of concern is not exceeded. All of the other hazard quotients are below a level of concern by a factor of at least 2 at the upper range of exposures and a factor of at least 10 at the central estimates of exposure. It should be noted that multiple applications of tebufenozide, such as those covered in this risk assessment, have no effect on the hazard quotients for workers. This is because all worker exposure assessments are based on the assumption that the worker applies the compound daily, albeit at different sites, over the course of an application season.

Mimic can cause eye irritation (section 3.1.11). Quantitative risk assessments for irritation are not derived; however, from a practical perspective, eye irritation is likely to be the only overt effect as a consequence of mishandling tebufenozide. This effect can be minimized or avoided by prudent industrial hygiene practices during the handling of the compound.

3.4.3. General Public

A quantitative summary of the risk characterization for members of the general public is presented in Worksheet E04 (Supplement 1). With the exception of the scenarios for the longer-term consumption of contaminated vegetation, all exposure scenarios are based on the highest application considered in this risk assessment – i.e., two applications at a rate of 0.12 lb/acre with an interval of 3 days between applications. Two scenarios are conducted for the longer-term consumption of contaminated vegetation, one involving two applications spaced three days apart and the other involving only a single application. Both are modeled at the maximum rate of 0.12 lb/acre. As with the risk characterization for workers, risk is expressed quantitatively as the hazard quotient using the surrogate acute RfD of 10 mg/kg (Section 3.3.3) for acute exposures and the chronic RfD of 0.018 mg/kg/day (Section 3.3.2) for longer-term exposures.

The only exposure scenario that leads to any unacceptable risk is the longer-tern consumption of contaminated vegetation. For two applications spaced three days apart at the maximum rate of 0.12 lb/acre, the hazard quotient 1.5 for the longer-term consumption of contaminated vegetation – i.e., the exposure exceeds the RfD by a factor of 1.5. Because the exposure is linearly related to the application rate, two exposures at an application rate of 0.08 lb/acre [0.12 lb/acre ÷ 1.5] would reach but not exceed the level of concern. With a single application at the maximum rate of 0.12 lb/acre, the hazard index is 0.8, below the level of concern. As discussed in Section 3.2.3.6, this exposure scenario assumes that an individual will consume over a 90 day period after that fruit had been directly sprayed. The probability of this occurring is unlikely because the USDA will not intentionally apply tebufenozide to crops or other food items. Nonetheless, this is a standard exposure scenario used in Forest Service risk assessments to consider the longer-term consumption of food items such as berries that might be sprayed during the broadcast application of a pesticide. This risk assessment suggests that two applications at 0.08 lb/acre or more should be avoided in areas where members of the general public might consume contaminated fruits.

None of the acute or other longer-term hazard quotients exceed 1 even at the upper ranges of

plausible exposure. The highest acute hazard quotient is 0.1, the upper range of risk for the consumption of contaminated water by child after an accidental spill. This extreme and accidental acute scenario is below the level of concern by a factor of 10. No other acute exposure scenarios, many of which involve extremely conservative assumptions, approach a level of concern at the upper range of exposure. Based on central estimates of exposure, which involve somewhat less conservative assumptions, the acute hazard quotients range from 0.00008 to 0.02 – i.e., below the level of concern by factors of 50 to 12,500. Based on central estimates of longer-term exposures, the hazard quotients range from 0.00003 to 0.03, below the level of concern by factors of about 30 to over 33,000.

3.4.4. Sensitive Subgroups

Some individuals are born with a form of congenital methemoglobinemia and may be at increased risk of adverse effects to compounds that induce methemoglobinemia (Centa et al. 1985; Das Gupta et al. 1980). Infants less than 3 months old have lower levels of methemoglobin (cytochrome b5) reductase and higher levels of methemoglobin (1.32%), compared with older children or adults (Centa et al. 1985; Smith 1996). A similar pattern is seen in many species of mammals (Lo and Agar 1986). Thus, it is possible that infants could be more sensitive to the effects of tebufenozide than adults.

3.4.5. Connected Actions

The most sensitive effect for tebufenozide, methemoglobinemia, is also associated with exposures to diflubenzuron, another agent used for gypsy moth control. These two agents are likely to have an additive effect on methemoglobinemia but these agents are not used together. Thus, simultaneous exposures are unlikely. Exposure to other compounds in the environment that induce methemoglobinemia may also lead to an additive effect. Any agent or condition that may reduce the oxygen carrying capacity of the blood could lead to increased risks from exposure to either tebufenozide or diflubenzuron. For example, individuals exposed to combustion smoke or carbon monoxide (that is, agents that do oxidative damage to blood) may be at increased risk of developing methemoglobinemia (Hoffman and Sauter 1989; Laney and Hoffman 1992). In addition, individuals exposed to high levels of nitrates, either in air or in water, will have increased levels of methemoglobin (Woebkenberg et al. 1981) and may be at increased risks of exposure to compounds such as tebufenozide.

3.4.6. Cumulative Effects

This risk assessment is based on two applications at the maximum allowable rate of 0.12 lb/acre. This approach is used to estimate maximum daily exposure and daily absorbed dose. In addition, this risk assessment specifically considers the effect of repeated exposure in that the chronic RfD is used as an index of acceptable longer-term exposures and an acute RfD based on an exposure period of 10 to 13 days is used for the risk characterization of single day exposures. Consequently, the risk characterizations presented in this risk assessment specifically addresses and encompasses the potential impact of long-term exposure and cumulative effects.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview. The toxicity of tebufenozide is well characterized in experimental mammals, birds, terrestrial invertebrates, and aquatic animals. Nonetheless, given the very large number of species in the environment which could be exposed to tebufenozide, toxicity data are available on relatively few species.

It seems reasonable to assume the most sensitive effects in wildlife mammalian species will be the same as those in experimental mammals (i.e., effects on the blood, specifically the formation of methemoglobin, which leads to a spectrum of other effects in blood that can be characterized as hemolytic anemia). At higher doses, tebufenozide was associated with impaired reproductive performance in experimental mammals, and this effect is also considered quantitatively in this risk assessment. Potential reproductive effects are also of concern for birds, although there are inconsistencies in the available experimental data. The available literature includes a reproduction study investigating effects in mallard ducks and two reproduction studies investigating effects in bobwhite quail. In one of the quail studies, dietary concentrations of 300 and 1000 ppm caused reproductive effects. These effects were not observed in that study at 100 ppm or in the more recent quail study or in the study on mallard ducks. A field study on the effects of tebufenozide on reproductive performance in birds noted trends that were statistically insignificant but suggestive of adverse reproductive effects in a warbler species. Thus, consistent with the interpretation by the U.S. EPA, reproductive effects in both mammals and birds are considered endpoints of concern in this risk assessment.

The mechanism of action of tebufenozide in target insects is relatively well understood. Tebufenozide mimics the action of the invertebrate hormone, 20-hydroxyecdysone, which controls molting. The effectiveness of tebufenozide in mimicking 20-hydroxyecdysone activity, however, appears to vary markedly among orders and species of invertebrates. In general, lepidopteran species are sensitive to tebufenozide but other insects are much less sensitive.

There are no bioassays regarding the toxicity of tebufenozide to terrestrial plants or terrestrial microorganisms in the literature. There are a number of field studies and field simulation studies available on tebufenozide and effects that might be associated with toxicity to plants or soil microorganisms have not been noted.

The acute toxicity of tebufenozide to aquatic animals is relatively low, with acute LC_{50} values ranging from 2.2 to 6.5 mg/L for fish and 0.3 to 3.8 mg/L for aquatic invertebrates. Nonetheless, much lower concentrations of tebufenozide may cause reproductive effects in fish (0.048 mg/L) and aquatic invertebrates (0.0053 mg/L).

4.1.2. Toxicity to Terrestrial Organisms.

4.1.2.1. Mammals– As summarized in the human health risk assessment (see Section 3.1), the mode of action of tebufenozide in mammals is relatively well characterized. Several standard toxicity studies in experimental mammals were conducted as part of the registration process (Appendix 2). The most sensitive effect in several species of experimental mammals involves effects on the blood, specifically the formation of methemoglobin, which leads to a spectrum of other effects in blood that can be characterized as hemolytic anemia. Since higher doses of tebufenozide were associated with impaired reproductive performance (see Section 3.1.4), both toxic and reproductive effects are considered in this risk assessment.

The acute toxicity of tebufenozide is relatively low, with an oral LD50 greater than 5000 mg/kg. The subchronic and chronic toxicity studies on tebufenozide were conducted in dogs, mice, and rats. The most sensitive effects involve changes to blood. The most sensitive species is the dog, with a NOAEL of 50 ppm in the diet (1.8 mg/kg bw/day) and an effect level of 500 ppm (about 20 mg/kg bw/day) over an exposure period of 1 year.

As discussed in Section 3.3.3, there is no apparent dose duration relationship for tebufenozide. In other words, short-term exposures are likely to lead to changes in the blood comparable to those observed after longer-term exposures. Thus, the chronic NOAEL of 1.8 mg/kg/day is used to characterize risks associated with both short- and long-term exposures.

4.1.2.2. Birds– Toxicity studies have been conducted on the acute toxicity and reproductive effects of tebufenozide in birds and a field study is available on reproductive effects.

Information regarding the laboratory tests on the toxicity of tebufenozide to birds is summarized in Appendix 4. The acute toxicity of tebufenozide is low for birds, as it is for mammals. When administered in gelatin capsules, the 21-day oral LD_{50} is greater than 2150 mg a.i./kg bw (Fletcher 1987). Similarly, in 5-day dietary studies, the dietary LC_{50} is greater than 5000 ppm (Fletcher 1990a,b). Hematological endpoints are not usually assayed in bioassays with birds, and there are no data regarding the hematological effects in birds after exposure to tebufenozide.

Nevertheless, the most relevant and significant studies for this risk assessment involve the potential reproductive effects in birds exposed to tebufenozide. Reproduction studies were conducted in mallard ducks (Beavers et al. 1993a) and bobwhite quail (Beavers et al. 1993b; Reinert 1995a). As indicated in Appendix 4, dietary concentrations less than or equal to 1000 ppm tebufenozide did not cause reproductive effects in mallard ducks In the quail studies, however, the results are inconsistent. In the earlier study by Beavers et al. (1993b), reproductive effects - including a reduced number of eggs laid, viable embryos and 14 day old survivors - were noted at dietary concentrations of 300 and 1000 ppm, but not at 100 ppm. In a similar study conducted later by Reinert (1995a), there were no substantial dose-related effects in quail exposed to dietary concentrations of up to 615 ppm.

In terms of the hazard identification, the most important question involves the extent to which

the Reinert (1995a) study reporting negative results for reproductive toxicity reduces the concerns raised by the Beavers et al. (1993b) study, which reports positive results. The earlier study was accepted by the U.S. EPA (1999e) and used in their ecological risk assessment of tebufenozide; however, the U.S. EPA (1999e) does not discuss the later negative study. The negative study is discussed in a review by Rohm and Haas (Keller and Brown 1998b), who question whether the NOAEL for the earlier study was 100 ppm or 300 ppm.

Regardless of which dose is classified as a NOAEL in the Beavers et al. (1993b) study, there seems to be no evidence that the study is flawed in any way. The minor differences between the early study and the later study, as detailed in Appendix 4, relate primarily to how exposures were reported and how food consumption was measured.

Notably, reproductive effects were observed also in mammals exposed to a dietary concentration of 2000 ppm (≈160 mg/kg bw), with a NOAEL of 150 ppm (≈12 mg/kg bw) (see Section 3.1.4). In the bobwhite quail study conducted by Beavers et al. (1993b), the dietary effect levels (AELs) of 300 and 1000 ppm correspond to estimated daily doses of 45 and 150 mg/kg/day, and the NOAEL of 100 ppm corresponds to an estimated daily dose of 15 mg/kg bw. Thus, the apparent NOAEL values and AEL values for mammals and birds are reasonably consistent. Finally, based on a metabolism study in hens (Sharma and Schuck 1996), the metabolic pathways for birds and mammals appear to be similar.

In the absence of any basis for discounting the earlier study in bobwhite quail (Beavers et al. 1993b) and given the reasonable consistency in dose levels associated with reproductive effects in mammals and birds as well as the similar metabolic pathways in mammals and birds, reproductive effects are considered an endpoint of concern in this risk assessment.

A field study on the reproductive performance of Tennessee warblers (*Vermivora peregrina*) in forests treated with Mimic has been published (Holmes 1998). In this study, Mimic was applied at a rate of 0.07 a.i. kg/ha, approximately 0.06 lb a.i./acre, in a forest area in Ontario. Two applications were made at this rate with a 4 day interval between applications. A number of reproductive parameters were assayed including number of eggs laid, percent hatch and growth of the hatchlings. There parameters were compared to an untreated control plot. A total of six nests were observed in the control plot and 5 nests in the plot treated with Mimic. No statistically significant adverse effects were noted. However, there were decreases in both the average number of eggs per nest (6.3 in the control area and 5.8 in the treated area) as well as the percent hatch (97.4% in the control area and 89.7% in the treated area). As noted by Holmes (1998, p. 191), the small sample sizes result in a low statistical power and the results are *"suggestive, although not necessarily compelling, that reproductive parameters were consistently lower in the treated blocks than in the control block."* Some differences in adult behavior were observed in the plot treated with Mimic – i.e., an increase in foraging time and an associated decrease in brooding time. This suggests that the primary effect on the birds may have been a decrease in food abundance.

This field study by Holmes (1998) combined with bobwhite quail assay conducted by Beavers et al. (1993b) raise concern that tebufenozide could cause adverse reproductive effects in birds. This concern is addressed quantitatively in this risk assessment for exposures involving the consumption of contaminated vegetation, fish, and insects.

4.1.2.3. Terrestrial Invertebrates – While Mimic is specifically used by the Forest Service for the control of the Gypsy moth, tebufenozide is effective in the control of other lepidopteran pest species, including the apple bud moth (*Platynota idaeusalis,* Biddinger et al. 1998), various species of spruce budworm (Cadogan et al. 1997; Payne et al. 1997; Retnakaran et al. 1997a,b), the tomato looper (*Deixis chalcites*, Smagghe et al. 1997), and the Indian-meal moth (*Plodia interpunctella*) (Oberlander et al. 1998). A complete list of the pest species for which tebufenozide is specified is provided in U.S. EPA (1999e).

The toxicity of tebufenozide has been assayed in several species (Appendix 5). The mechanism of action of tebufenozide in target insects is relatively well understood. In sensitive species, tebufenozide mimics the action of the invertebrate hormone 20-hydroxyecdysone. This hormone controls molting in insects and various terrestrial and aquatic invertebrates, which is mediated through binding to species-specific ecdysone receptors present in the cytoplasm of epidermal cells (Addison 1996; Keller 1998; Smagghe and Degheele 1994a; U.S. EPA 1999e).

While 20-hydroxyecdysone is a hormone common to many invertebrates, the effectiveness of tebufenozide in mimicking 20-hydroxyecdysone activity seems to vary markedly among orders and species of invertebrates. Although the specificity of tebufenozide is not addressed in detail in the recent U.S. EPA (1999e) ecological risk assessment, it was reviewed in detail by Rohm and Haas (Keller 1998). The review by Keller (1998) is consistent with publications in the open literature relating to species specificity of tebufenozide (Addison. 1996; Biddinger and Hull. 1995; Biddinger et al. 1998; Brown. 1996; Butler et al. 1997; Dhadialla et al. 1998; Rumpf et al. 1998; Smagghe and Degheele 1994a,b, 1997; Smagghe et al. 1995, 1996a,b; Valentine et al. 1996). In general, *Lepidoptera* are sensitive to tebufenozide but other insects are much less sensitive (Smagghe and Degheele 1994a). The differences in sensitivity appear to be related to differences in ecdysone receptor binding (Smagghe et al. 1996a) rather than differences in pharmacokinetics (Smagghe and Degheele 1994b).

There are four studies regarding the effects of tebufenozide to terrestrial invertebrates under field or field simulation conditions (Appendix 6). Three of these studies are published in the open literature (Addison 1996; Butler et al. 1997; Valentine et al. 1996), and one unpublished study was conducted by Rohm and Haas (Walgenbach 1995). The studies by Addison (1996) and Butler et al. (1997) are most directly relevant to this risk assessment because they assayed the effects on nontarget invertebrates in the forest canopy (Butler et al. 1997) and forest soil (Addison 1996) after the application of tebufenozide.

In the study by Addison (1996), tebufenozide was incorporated into forest soil at a concentration of 72.1 ppm. Based on a typical application rate of 70 g/ha and the assumption that tebufenozide

will remain in the top 2 cm of soil, Addison (1996) estimated that the soil concentration of 72.1 ppm is equivalent to a concentration that is 100 times greater than expected environmental concentrations. There were no adverse effects on one species of earthworm (*Dendrobaena octaedra*) or on four species of Colembola (*Folsomia candida*, *Folsomia nivalis*, *Onychiurus parvicornis*, and *Hypogastrura pannosa*), which are indigenous to forest soils in Canada and the northern United States. Consistent with results of the Addison (1996) study, a standard bioassay on earthworms (*Eisenia foetida*) noted no adverse effects at soil concentrations of up to 1000 ppm over a 14-day exposure period (Garvey 1992).

Butler et al. (1997) conducted a study on canopy arthropods in which Mimic 4F was applied at rates of 0.03 and 0.06 lb a.i./acre to a mixed oak plot in Ohio. The investigators examined Mimic's efficacy against Gypsy moth larvae and its effects on nontarget arthropods. Population assays included measures of abundance and diversity in10 arthropod families and 15 lepidopteran species. No effects on abundance or richness were noted in any organisms other than lepidopteran species. A decrease in abundance was noted in some lepidopteran species. The study indicates that there were problems associated with the application of Mimic 4F that resulted in poorer than expected efficacy, and that consequently, effects in nontarget lepidopteran species may have been underestimated.

The studies by Valentine et al. (1996) and Walgenbach (1995) involve the application of tebufenozide formulations to apple orchards. The study by Valentine et al. (1996) found no effects of tebufenozide on species of mites, spiders, various beetles (*Coleoptera*), and true bugs (*Hemiptera*) after Mimic was applied to apple orchards at rates that were effective in controlling lepidopteran pest species. Similarly, Walgenbach (1995) noted no effects on beneficial insect populations after Confirm was applied to apple plots. While not as directly relevant to this risk assessment as the forestry studies summarized above, these two studies support the general conclusion that tebufenozide is likely to have an adverse impact on *Lepidoptera* but not on non-lepidopteran species.

In addition to the above studies, the standard bee toxicity assay was conducted on tebufenozide (Atkins. 1990; Chan 1995). In this study, no mortality was observed at doses of up to 233.98 µg a.i./bee. Using a body weight of 0.093 g for the honey bee (USDA/APHIS 1993), this corresponds to a dose of about 2500 mg/kg bw [0.23 mg/0.000093 kg = 2473 mg/kg bw].

4.1.2.4. Terrestrial Plants (Macrophytes)– Standard bioassays for toxicity to terrestrial plants are required by the U.S. EPA for the registration of herbicides but not insecticides. No bioassays for herbicidal activity of tebufenozide were encountered in the published literature or in the U.S. EPA/OPP files. Thus, the potential effects of tebufenozide on terrestrial plant species is not discussed in other reviews of this compound (U.S. EPA 1999d,e; Keller 1998). The implicit presumption is that plausible levels of exposure to tebufenozide will not adversely affect terrestrial plant species.

There are several field studies regarding the efficacy of tebufenozide applied to terrestrial

vegetation for the control of various insect pests (e.g., Biddinger et al. 1998; Cadogan et al. 1997; Oberlander et al. 1998; Payne et al. 1997; Retnakaran et al. 1997a,b; Valentine et al. 1996; West et al. 1997). If tebufenozide were toxic to terrestrial plants at application rates that are used in the field, it is plausible that adverse effects would be reported in this literature. No such reports were encountered.

Because there is no basis for further evaluating the assumption that tebufenozide will not cause adverse effects in terrestrial plants, such effects will not be considered quantitatively in this risk assessment.

4.1.2.5. Terrestrial Microorganisms– As indicated in U.S. EPA (1999e), microbial transformation is the predominant route of environmental degradation in soil and water. Data regarding the toxicity of tebufenozide to terrestrial microorganisms, as with terrestrial plants, is not available in the open literature or the U.S. EPA/OPP files. Tebufenozide is degraded in soil by some microorganisms (e.g., Sundaram 1996, 1997a). Nonetheless, given the diversity of soil microorganisms and soil environments, generalizations concerning the potential effects on soil microflora cannot be supported.

4.1.3. Aquatic Organisms.

4.1.3.1. Fish– Information on the toxicity of tebufenozide to fish is summarized in Appendix 7. All of the available studies were conducted in support of the registration of tebufenozide and submitted to U.S. EPA/OPP. The summaries of these studies given in Appendix 7 were taken from the full text copies of the studies submitted to U.S. EPA.

The acute toxicity of tebufenozide to fish is relatively low – i.e., LC_{50} values of 3.0 mg a.i./L in Bluegill sunfish (Graves and Smith 1992b) and 5.7 mg a.i./L in Rainbow trout (Graves and Smith 1992c). There is greater concern, however, regarding the potential chronic toxicity of tebufenozide to fish. The U.S. EPA evaluates all studies like those summarized in Appendix 7 to determine whether the conclusions from the studies are consistent with the data presented in the studies. In many instances, the U.S. EPA accepts the study conclusions. For tebufenozide, however, the U.S. EPA has disagreed with conclusions for a fathead minnow egg and fry study (Bettancourt 1992) as well as a fathead minnow full life cycle study (Rhodes and Leak 1996). This is discussed further in the dose-response assessment (Section 4.3.3.1).

4.1.3.2. Amphibians– No information was encountered on the toxicity of tebufenozide to amphibians.

4.1.3.3. Aquatic Invertebrates – Unpublished studies on the toxicity of tebufenozide to aquatic invertebrates that were submitted to the U.S. EPA in support of the registration of tebufenozide are summarized in Appendix 8. Some invertebrate assays were conducted in support of the registration of tebufenozide, and the summaries of these studies are based on the full text copies of the studies submitted to U.S. EPA. Additional studies published in the open literature are discussed below. Unlike some of the fish studies, the studies on aquatic invertebrates,

summarized in Appendix 8, were accepted without exception by the U.S. EPA (1999e).

In the studies submitted for registration, the acute toxicity of tebufenozide to daphnia (*Crustacea*) and midges (*Insecta*) is on the same order as that for fish, with a 48 hour LC_{50} value of 3.8 mg/L for daphnids (Graves and Smith 1992a) and a 96 hour LC_{50} value of 0.3 mg/L for midge larvae (van der Kolk 1997). Similarly, in a study published in the open literature and sponsored by the U.S. Geological survey, Song et al. (1997) report higher LC_{50} values for Crustacea (daphnia = 17.37 mg/L; Artemia = 5.53 mg/L) than for two species of mosquitoes (0.92 mg/L for *Aedes aegypti* and 0.15 mg/L for *Aedes taeniorhynchus*). All of these bioassay results from Song et al. (1997) involved exposures at 27°C. In similar bioassays conducted at 20°C, tebufenozide was substantially less toxic to both daphnids and *Aedes aegypti*. This negative relationship between toxicity and temperature is common.

As with fish, there is a concern for potential reproductive effects in both a free swimming species (*Daphnia*) as well as a sediment dwelling species (midge). In *Daphnia magna*, significant decreases in the number of offspring/female were noted at 0.12 mg/L and a significant decrease in the growth of offspring was noted at 0.059 mg/L (McNamara 1991). In midges (*Chironomus riparius*), a decrease in larval emergence was noted at a concentration of 0.0053 mg/L. At concentrations of 0.04 mg/L and higher, midge emergence was completely suppressed (van der Kolk 1997).

Kreutzweiser and Thomas (1995) assayed the effects of tebufenozide on aquatic invertebrate communities in lake enclosures at nominal concentrations of 0.07, 0.13, 0.33, and 0.66 mg/L. A dose-related decrease in cladoceran abundance was noted and persisted for 1-2 months at the two lower concentrations and for 12-13 months at the two higher concentrations. The decrease in cladoceran abundance was accompanied by an increase in the abundance of rotifers, suggesting that the changes in community structure could be attributable to secondary or trophic effects rather than to toxicity.

Rohm and Haas summarized the results of Kreutzweiser and Thomas (1995) along with several other field studies or field simulation studies (e.g. Kreutzweiser et al. 1994) regarding the effects of tebufenozide to aquatic invertebrates (Keller 1998). The most relevant study for this risk assessment is an unpublished report submitted to U.S. EPA (Russell et al. 1996). In this study, Mimic was applied at a rate of 70 g a.i./ha to a small forest pond. The application resulted in an initial concentration of 0.00837 mg/L which decreased to 0.00016 mg/L 1 month after spray. During the 1-month post-application observation period, no adverse effects were noted on invertebrate populations, compared with a control (untreated) pond. Notably, the maximum concentration of 0.00837 mg/L is very close to the effect level of 0.0053 mg/L for midge larvae; however, the average concentration during the 1-month study was probably substantially below the effect level in midges. Thus, although this study seems to support the assertion that tebufenozide can be applied without interfering with aquatic invertebrate communities, it is not in conflict with the available bioassay data.

4.1.3.4. Aquatic Plants – The toxicity of tebufenozide was assayed in two species of freshwater green algae, and details of these studies are presented in Appendix 8 along with the studies on aquatic invertebrates. *Selenastrum capricornutum* appears to be relatively insensitive to tebufenozide, with a NOEC for reduced cell density of 0.64 mg/L (Reinert 1993b), which is greater than the effect levels in aquatic invertebrates by a factor of 10-100.

Scenedesmus subspicatus appears to be much more sensitive than *Selenastrum capricornutum* although still much less sensitive than aquatic invertebrates, with a NOAEL and LOAEL for growth rate inhibition of 0.077 and 0.15 mg/L, respectively. Decreased cell density was a somewhat more sensitive effect with a NOAEL 0.046 mg/L and a LOAEL of 0.077 mg/L (Hoberg 1992a).

In an aquatic microcosm study with mixed species of algae, Sundaram et al. (1997b) report that tebufenozide stimulated algal growth at concentrations of 0.25 and 0.75 mg/L.

4.1.3.5. Aquatic Microorganisms (Other than algae) – Other than the effect in algae, summarized in the previous section, no studies regarding the toxicity of tebufenozide to aquatic microorganisms were encountered.

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview

Details of the exposure assessments for tebufenozide are given in the EXCEL workbook that accompany this risk assessment (Supplement 1). Most exposure assessments are based on two applications spaced 3 days apart at an application rate of 0.12 lb/acre. As in the human health risk assessment, two sets of exposure assessments are given for scenarios involving the longer-term consumption of contaminated vegetation: one for a single application at 0.12 lb/acre and another for two applications spaced 3 days apart at an application rate of 0.12 lb/acre.

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. For tebufenozide, the highest acute exposure for a terrestrial vertebrate is associated with a fish-eating bird and could reach up to about 85 mg/kg. Exposures anticipated from the consumption of contaminated vegetation by terrestrial animals range from central estimates of about 0.15 mg/kg for a small mammal consuming fruit to about 3 mg/kg for a large bird with upper ranges of about 0.4 mg/kg for a small mammal and 9 mg/kg for a large bird. The consumption of contaminated water leads to much lower levels of exposure. A similar pattern is seen for chronic exposures. Estimated longer-term daily doses for the a small mammal from the consumption of contaminated vegetation at the application site are in the range of about 0.000002 mg/kg/day to 0.08 mg/kg/day. Large birds feeding on contaminated vegetation at the application site could be exposed to much higher concentrations, ranging from about 0.015 mg/kg/day to 11 mg/kg/day. The upper ranges of exposure from contaminated vegetation far exceed doses that are anticipated from the consumption of contaminated water, which range from about 0.0000003 mg/kg/day to 0.0002 mg/kg/day for a small mammal.

Exposure to aquatic organisms is based on essentially the same information used to assess the exposure to terrestrial species from contaminated water. The peak estimated concentration of tebufenozide in ambient water is 10 (0.005 to 40) µg/L after two applications of 0.12 lb/acre spaced three days apart. For longer-term exposures, the corresponding longer term concentrations in ambient water are estimated at about 0.004 (0.00002 to 0.01) µg/L.

4.2.2. Terrestrial Animals

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.

In this exposure assessment, estimates of oral exposure are expressed in the same units as the available toxicity data. As in the human health risk assessment, these units are usually expressed as mg of agent per kg of body weight and abbreviated as mg/kg for terrestrial animals. One exception in this risk assessment involves terrestrial invertebrates. As detailed in the dose-response assessment (Section 4.3), toxicity data in units of mg/kg bw are available for some terrestrial invertebrates and these data are used in a manner similar to that for terrestrial vertebrates. For other species, however, standard toxicity studies report units that are not directly

useful in a quantitative risk assessments – e.g., contact toxicity based on petri dish exposures. As an alternative, some dose response assessments are based on field studies in which the dose metameter is simply the application rate in units of mass per area such as g a.i./ha.

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.

The exposure assessments for terrestrial animals are summarized in Worksheet G01. As with the human health exposure assessment, the computational details for each exposure assessment presented in this section are provided as scenario specific worksheets (Worksheets F01 through F16b). Given the large number of species that could be exposed to insecticides and the varied diets in each of these species, a very large number of different exposure scenarios could be generated. For this generic risk assessment, an attempt is made to limit the number of exposure scenarios.

Because of the relationship of body weight to surface area as well as to the consumption of food and water, small animals will generally receive a higher dose, in terms of mg/kg body weight, than large animals will receive for a given type of exposure. Consequently, most general exposure scenarios for mammals and birds are based on a small mammal or bird. For mammals, the body weight is taken as 20 grams, typical of mice, and exposure assessments are conducted for direct spray (F01 and F02a), consumption of contaminated fruit (F03, F04a, F04b), and contaminated water (F05, F06, F07). Grasses will generally have higher concentrations of insecticides than fruits and other types of vegetation (Fletcher et al. 1994; Hoerger and Kenaga 1972). Because small mammals do not generally consume large amounts of grass, the scenario for the assessment of contaminated grass is based on a large mammal (Worksheets F10, F11a, and F11b). Other exposure scenarios for a mammals involve the consumption of contaminated insects by a small mammal (Worksheet F14a) and the consumption of small mammals contaminated by direct spray by a large mammalian carnivore (Worksheet F16a). Exposure scenarios for birds involve the consumption of contaminated insects by a small bird (Worksheet F14b), the consumption of contaminated fish by a predatory bird (Worksheets F08 and F09), the consumption by a predatory bird of small mammals contaminated by direct spray and the consumption by a large bird of contaminated grasses (F12, F13a, and F13b).

While a very large number of other exposure scenarios could be generated, the specific exposure scenarios developed in this section 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.

4.2.2.1. 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.

For this risk assessment, three groups of direct spray exposure assessments are conducted. The first, which is defined in Worksheet F01, involves a 20 g mammal that is sprayed directly over one half of the body surface as the chemical is being applied. The range of application rates as well as the typical application rate is used to define the amount deposited on the organism. The absorbed dose over the first day (i.e., a 24-hour period) is estimated using the assumption of first-order dermal absorption. An empirical relationship between body weight and surface area (Boxenbaum and D'Souza 1990) is used to estimate the surface area of the animal. The estimates of absorbed doses in this scenario may bracket plausible levels of exposure for small mammals based on uncertainties in the dermal absorption rate.

Other, perhaps more substantial, uncertainties affect the estimates for absorbed dose. 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, some animals, particularly birds and mammals, groom frequently, and grooming may contribute to the total absorbed dose by direct ingestion of the compound residing on fur or feathers. Furthermore, other vertebrates, particularly amphibians, may have skin that is far more permeable than the skin of most mammals. Quantitative methods for considering the effects of grooming or increased dermal permeability are not available. As a conservative upper limit, the second exposure scenario, detailed in Worksheet F02a, is developed in which complete absorption over day 1 of exposure is assumed.

Because of the relationship of body size to surface area, very small organisms, like bees and other terrestrial invertebrates, might be exposed to much greater amounts of a pesticide per unit body weight compared with small mammals. Consequently, a third exposure assessment is developed using a body weight of 0.093 g for the honey bee (USDA/APHIS 1993) and the equation above for body surface area proposed by Boxenbaum and D'Souza (1990). Because there is no information regarding the dermal absorption rate of tebufenozide by bees or other invertebrates, this exposure scenario, detailed in Worksheet F02b, also assumes complete absorption over the first day of exposure. As noted above, exposures for other terrestrial invertebrates are based on field studies in which application rate is the most relevant expression of exposure. This is discussed further in Section 3.3 (Dose-Response Assessment) and Section 3.4 (Risk Characterization).

Direct spray scenarios are not given for large mammals. As noted above, allometric relationships dictate that large mammals will be exposed to lesser amounts of a compound in any direct spray scenario than smaller mammals.

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

4.2.2.3. Ingestion of Contaminated Vegetation or Prey – Since tebufenozide will be applied to vegetation, the consumption of contaminated vegetation is an obvious concern and separate exposure scenarios are developed for acute and chronic exposure scenarios for a small mammal (Worksheets F04a and F04b) and large mammal (Worksheets F10, F11a, and F11b) as well as large birds (Worksheets F12, F13a, and F13b).

As discussed in Section 2.4, tebufenozide may be applied once or twice per season at an application rate of up to 0.12 lb/acre per application. In order to encompass the effects of both a single application per season and two applications per season, two sets of exposure assessments are given for the all scenarios involving the longer-term consumption of contaminated vegetation: one for a single application at 0.12 lb/acre and another for two applications spaced 3 days apart at an application rate of 0.12 lb/acre. For example, Worksheet 04bi presents the time-weighted average dose for a single application and Worksheet 04bii presents the time-weighted average dose for two applications spaced 3 days apart. This is also done for Worksheets F11a, F11b, F13a, and F13b. The calculation of the time-weighted average doses are identical to those used in the human health risk assessment (Section 3.2.3.6).

For the consumption of contaminated vegetation, a small mammal is used because allometric relationships indicate that small mammals will ingest greater amounts of food per unit body weight, compared with large mammals. The amount of food consumed per day by a small mammal (i.e., an animal weighing approximately 20 g) is equal to about 15% of the mammal's total body weight (U.S. EPA/ORD 1989). When applied generally, this value may overestimate or underestimate exposure in some circumstances. For example, a 20 g herbivore has a caloric requirement of about 13.5 kcal/day. If the diet of the herbivore consists largely of seeds (4.92 kcal/g), the animal would have to consume a daily amount of food equivalent to approximately 14% of its body weight [(13.5 kcal/day ÷ 4.92 kcal/g)÷20g = 0.137]. Conversely, if the diet of the herbivore consists largely of vegetation (2.46 kcal/g), the animal would have to consume a

daily amount of food equivalent to approximately 27% of its body weight [(13.5 kcal/day ÷ 2.46 kcal/g)÷20g = 0.274] (U.S. EPA/ORD 1993, pp.3-5 to 3-6). For this exposure assessment (Worksheet F03), the amount of food consumed per day by a small mammal weighing 20 g is estimated at about 3.6 g/day or about 18% of body weight per day from the general allometric relationship for food consumption in rodents (U.S. EPA/ORD 1993, p. 3-6).

A large herbivorous mammal is included because empirical relationships of concentrations of pesticides in vegetation, discussed below, indicate that grasses may have substantially higher pesticide residues than other types of vegetation such as forage crops or fruits (Worksheet A04). Grasses are an important part of the diet for some large herbivores, but most small mammals do not consume grasses as a substantial proportion of their diet. Thus, even though using residues from grass to model exposure for a small mammal is the most conservative approach, it is not generally applicable to the assessment of potential adverse effects. Hence, in the exposure scenarios for large mammals, the consumption of contaminated range grass is modeled for a 70 kg herbivore. Caloric requirements for herbivores and the caloric content of vegetation are used to estimate food consumption based on data from U.S. EPA/ORD (1993). Details of these exposure scenarios are given in worksheets F10 for acute exposures as well as Worksheets F11a and F11b for longer-term exposures.

For the acute exposures, the assumption is made that the vegetation is sprayed directly – i.e., the animal grazes on site – and that 100% of the animal's diet is contaminated. While appropriately conservative for acute exposures, neither of these assumptions are plausible for longer-term exposures. Thus, for the longer-term exposure scenarios for the large mammal, two sub-scenarios are given. The first is an on-site scenario that assumes that a 70 kg herbivore consumes short grass for a 90 day period after application of the chemical. In the worksheets, the contaminated vegetation is assumed to account for 30% of the diet with a range of 10% to 100% of the diet. These are essentially arbitrary assumptions reflecting grazing time at the application site by the animal. Because the animal is assumed to be feeding at the application site, drift is set to unity - i.e., direct spray. This scenario is detailed in Worksheet 11a. The second sub-scenario is similar except the assumption is made that the animal is grazing at distances of 25 to 100 feet from the application site (lowing risk) but that the animal consumes 100% of the diet from the contaminated area (increasing risk). For this scenario, detailed in Worksheet F12b, AgDRIFT is used to estimate deposition on the off-site vegetation. Drift estimates from AgDrift are summarized in Worksheet A06 and this model is discussed further in Section 4.2.3.2.

The consumption of contaminated vegetation is also modeled for a large bird. For these exposure scenarios, the consumption of range grass by a 4 kg herbivorous bird, like a Canada Goose, is modeled for both acute (Worksheet F12) and chronic exposures (Worksheets F13a and F13b). As with the large mammal, the two chronic exposure scenarios involve sub-scenarios for on-site as well as off-site exposure.

For this component of the exposure assessment, the estimated amounts of pesticide residue in vegetation are based on the relationship between application rate and residue rates on different

types of vegetation. As summarized in Worksheet A04, these residue rates are based on estimated residue rates from Fletcher et al. (1994).

Similarly, the consumption of contaminated insects is modeled for a small (10g) bird and a small (20g) mammal. No monitoring data have been encountered on the concentrations of tebufenozide in insects after applications of tebufenozide. The empirical relationships recommended by Fletcher et al. (1994) are used as surrogates as detailed in Worksheets F14a and F14b. To be conservative, the residue rates from small insects are used – i.e., 45 to 135 ppm per lb/ac – rather than the residue rates from large insects – i.e., 7 to 15 ppm per lb/ac.

A similar set of scenarios is provided for the consumption of small mammals by either a predatory mammal (Worksheet 16a) or a predatory bird (Worksheet 16a). Each of these scenarios assumes that the small mammal is directly sprayed at the specified application and the concentration of the compound in the small mammal is taken from the worksheet for direct spray of a small mammal under the assumption of 100% absorption (Worksheet F02a).

In addition to the consumption of contaminated vegetation and insects, tebufenozide may reach ambient water and fish. Thus, a separate exposure scenario is developed for the consumption of contaminated fish by a predatory bird in both acute (Worksheet F08) and chronic (Worksheet F09) exposures. Because predatory birds usually consume more food per unit body weight than do predatory mammals (U.S. EPA 1993, pp. 3-4 to 3-6), separate exposure scenarios for the consumption of contaminated fish by predatory mammals are not developed.

4.2.2.4. Ingestion of Contaminated Water – Estimated concentrations of tebufenozide in water are identical to those used in the human health risk assessment (Worksheet B06). The only major differences involve the weight of the animal and the amount of water consumed. There are well-established relationships between body weight and water consumption across a wide range of mammalian species (e.g., U.S. EPA 1989). Mice, weighing about 0.02 kg, consume approximately 0.005 L of water/day (i.e., 0.25 L/kg body weight/day). These values are used in the exposure assessment for the small (20 g) mammal. Unlike the human health risk assessment, estimates of the variability of water consumption are not available. Thus, for the acute scenario, the only factors affecting the estimate of the ingested dose include the field dilution rates (i.e., the concentration of the chemical in the solution that is spilled) and the amount of solution that is spilled. As in the acute exposure scenario for the human health risk assessment, the amount of the spilled solution is taken as 200 gallons. In the exposure scenario involving contaminated ponds or streams due to contamination by runoff or percolation, the factors that affect the variability are the water contamination rate, (see Section 3.2.3.4.2) and the application rate. Details regarding these calculations are summarized in Worksheets F06 and Worksheet F07.

4.2.3. Terrestrial Plants

Terrestrial plants will certainly be exposed to tebufenozide. 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 tebufenozide, however, the development of such exposure assessments would serve no purpose. As discussed in Section 4.1.2.4 (Hazard Identification for Terrestrial Plants), there is no basis for asserting that tebufenozide will cause adverse effects in terrestrial plants. Thus, no formal exposure assessment is conducted for terrestrial plants.

4.2.4. Soil Organisms

For both soil microorganisms and soil invertebrates, the toxicity data are typically expressed in units of soil concentration – i.e., mg agent/kg soil which is equivalent to parts per million (ppm) concentrations in soil. The GLEAMS modeling, discussed in Section 3.2.3.4.3, provides estimates of concentration in soil as well as estimates of off-site movement (runoff, sediment, and percolation). Based on the GLEAMS modeling, concentrations in clay, loam, and sand over a wide range of rainfall rates are summarized in Table 4-1. As indicated in this table, peak soil concentrations after two applications at an application rate of 0.12 lb/acre are in a relatively narrow range: about 0.02 to 0.1 mg/kg (ppm) over all soil types and rainfall rates. Longer term concentrations in soil are all low and are on the order of 0.003 to 0.05 mg/kg – i.e., 3 ppb to 50 ppb.

4.2.5. Aquatic Organisms

The plausibility of effects on aquatic species is based on estimated concentrations of tebufenozide in water that are identical to those used in the human health risk assessment. As summarized in Table 3-5, the peak estimated concentration of tebufenozide in ambient water is 10 (0.005 to 40) µg/L after two applications of 0.12 lb/acre spaced three days apart. For longer-term exposures, the corresponding longer term concentrations in ambient water are estimated at about 0.004 (0.00002 to 0.01) µg/L.

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Overview

The specific toxicity values used in this risk assessment are summarized in Table 4-2, and the derivation of each of these values is discussed in the various subsections of this dose-response assessment. The first column in Table 4-2 specifies the organism to which the toxicity value applies. The available toxicity data support separate dose-response assessments in six classes of organisms: terrestrial mammals, birds, nontarget terrestrial invertebrates, fish, aquatic invertebrates, and aquatic algae. Different units of exposure are used for different groups of organisms depending on how exposures are likely to occur and how the available toxicity data are expressed.

Tebufenozide is relatively non-toxic to mammals and birds. For mammals, the toxicity values used in the ecological risk assessment are identical to those used in the human health risk assessments: an acute NOAEL for reproductive toxicity of 1000 mg/kg and a chronic NOAEL of 1.8 mg/kg/day based on effects on the blood. For birds, the acute NOAEL for tebufenozide is taken as 2150 mg/kg from an acute oral study in which the dose was administered in capsules for 21-days. The longer term NOAEL is taken as 15 mg/kg/day from a standard reproduction study in bobwhite quail.

For terrestrial invertebrates, three types of data are used to characterize risks: a contact bioassay in the honey bee, a soil bioassay in earthworms, and field studies in which population level effects were monitored in insects. The standard contact bioassay in honey bees indicates an NOEC of 2500 mg/kg bw, comparable to the acute toxicity values in mammals and birds. The earthworm bioassay indicates a NOEC of 1000 mg/kg soil. The available field studies indicate that tolerant insect species are not affected by application rates up to 0.24 lb/acre. The true NOEC may be higher – i.e., an LOEC has not been identified for tolerant species of terrestrial insects. Conversely, application rates as low as 0.03 lb/acre have been shown to adversely affect sensitive nontarget insects, primarily *Lepidoptera* and a NOEC for sensitive species has not been identified.

Acute toxicity values for aquatic species indicate relatively little difference between fish and aquatic invertebrates. For fish, the acute NOEC values are 0.39 mg/L and 1.9 mg/L for sensitive and tolerant species, respectively. For invertebrates, the corresponding acute NOEC values are 0.12 mg/L and 0.82 mg/L. Differences between fish and invertebrates are difficult to assess in terms of longer-term toxicity. For fish, data are available on only a single species, the fathead minnow, and only a LOAEL of 0.048 mg/L is available. For invertebrates, longer-term NOEC values of 0.0035 mg/L and 0.029 mg/L are used for sensitive and tolerant species. Toxicity values for aquatic plants are taken as 0.077 mg/L for sensitive species and 0.64 mg/L for tolerant species, somewhat below the acute NOEC values in fish and aquatic invertebrates. Because of the short life-cycle of individual algal cells, the relatively short-term bioassays in algae (i.e., 96 to 120 hours) are applied to both acute and longer-term concentrations for the characterization of risk.

4.3.2. Toxicity to Terrestrial Organisms

4.3.2.1. Mammals – As summarized in the dose-response assessment for the human health risk assessment (see Section 3.3.3.), the most sensitive effect in experimental mammals involves toxic effects in red blood cells. The chronic NOAEL for this endpoint in experimental mammals is 1.8 mg/kg/day (U.S. EPA 1999b) and is based on a dog study (Richards 1992a) in which beagles of either sex were provided with dietary concentrations of 0, 15, 50, 250, or 1500 ppm technical grade tebufenozide for 52 weeks (Appendix 2). No effects were seen in the 50 ppm exposure group which corresponded to an average dose of 1.8 mg/kg/day. At 250 ppm, which corresponded to an average dose of 20 mg/kg/day, a direct effect on red blood cells was indicated by increased concentrations of methemoglobin in the blood as well as changes in several other hematological parameters associated with toxic effects in red blood cells. Thus, for this risk assessment, 1.8 mg/kg/day is taken as the chronic NOAEL for general toxic effects.

Tebufenozide is also associated with adverse reproductive effects in mammals in a 2-generation study (see Section 3.1.4). In the study by Danberry et al. (1993), reproductive effects were not observed in rats given a dietary concentration of 150 ppm (≈ 12 mg/kg bw) tebufenozide; however, in the same study, rats given a dietary concentration of 2000 ppm (≈ 160 mg/kg bw) demonstrated clearly adverse effects, including increased mortality in females during delivery and decreases in implantation. This endpoint, with a longer-term NOAEL of 12 mg/kg/day and a LOAEL of 160 mg/kg/day, is also used in the characterization of risk (Section 4.4.2) to help elaborate the potential effects of exposures that exceed the general NOAEL of 1.8 mg/kg/day.

Consistent with the approach taken in the human health risk assessment (Section 3.3.4), acute (1-day) exposures will be based on the acute NOAEL of 1000 mg/kg/day from reproduction studies in both rats (Hoberman 1991) and rabbits (rabbits) involving 10 to 13 day exposure periods.

4.3.2.2. Birds – As detailed in Appendix 4, adverse reproductive effects were observed in bobwhite quail provided with dietary concentrations of 300 or 1000 ppm (Beavers et al. 1993b). Similar effects were not observed in mallard ducks provided with dietary concentrations of up to 1000 ppm in a study conducted by the same investigators (Beavers et al. 1993a) or in a follow-up study on bobwhite quail provided with dietary concentrations of up to 615 ppm (Reinert 1995a). As discussed in Section 4.1.2.2, the earlier study by Beavers et al. (1993b) is used to identify reproductive toxicity as an endpoint of concern in this risk assessment because there is no basis for discounting the study or explaining the discrepancies between the Beavers et al. (1993b) and Reinert (1995a) studies in bobwhite quail. In addition, reasonable consistency is apparent in the reported dose levels associated with reproductive effects in mammals and the reported dose levels in Beavers et al. (1993b) study. This approach is consistent with that taken by U.S. EPA (1999e).

It is worth noting that the two quail studies use different methods to report the estimated dose (i.e., the dose as mg/kg bw/day based on dietary concentrations and food consumption). In the study by Beavers et al. (1993b), "No attempt was made to quantify the amount of feed wasted by

the birds, as the wasted feed is normally scattered and mixed with water and excreta." (Beavers et al. 1993b, p. 16). In the study by Reinert (1995a), food consumption estimates did explicitly consider measurements of food wastage (i.e., food scattered from the container and not consumed). Furthermore, the study by Beavers et al. (1993b) states explicitly that food was administered *ad libitum*—an excess of food was freely available to the animals. This protocol is not specified in the study by Reinert (1995a); however, it seems reasonable to assume that the food was available *ad libitum* because a restricted feeding protocol is atypical and would have been specified in the methods section of the study. These reporting differences are relatively inconsequential, assuming that both studies use *ad libitum* feeding.

Of greater importance, however, is the exposure metameter (i.e., how the exposure is expressed in the dose-response and the exposure assessments). The U.S. EPA (1999e) uses reported dietary concentrations. This approach, however, may be under protective. Laboratory diets generally involve the use of dry food, and dry food is specified in all of the bird feeding studies on tebufenozide. Dry laboratory chow usually has a higher caloric content than food consumed in the wild, if only because most food consumed in the wild has a high water content. In addition, most reported concentrations of a pesticide in environmental samples are given on a wet (natural) weight rather than a dry (dedicated) weight basis. Consequently, animals tend to eat greater amounts of food in the wild than they do under laboratory conditions (U.S. EPA 1993). Consequently, for a fixed concentration in food, ingested doses expressed as mg/kg bw/day often will be higher in free living animals than in laboratory animals.

Because of these relationships, Forest Service risk assessments use doses expressed as mg/kg body weight for both the exposure and dose-response assessments. As detailed in the worksheets, information on caloric requirements and caloric values of different foods are used to estimate the amount of a particular food that an animal will use.

For this risk assessment, the food consumption values reported by Beavers et al. (1993b) are used to estimate a NOAEL and a LOAEL of 15 and 45 mg/kg bw/day, respectively. This is not the most conservative approach that could be taken, because Beavers et al. (1993b) did not consider wastage in their estimates of food consumption. By comparison with the study by Reinert (1995a), the food consumption and hence the ingested amounts of tebufenozide could have been lower by a factor of about 2 [i.e., food consumption rates of 30 g per bird in Beavers et al. (1993b) and 16 g per bird in Reinert (1995a)]. Compared with other uncertainties in this risk assessment, this difference is relatively modest. The dose adjustment is incorporated explicitly into the dose-response assessment, and given further consideration in the risk characterization.

As with mammals, the acute toxicity of tebufenozide to birds appears to be very low. As indicated in Appendix 4, acute dietary LC_{50} values are greater than 5000 ppm (mg tebufenozide per kg diet) in both bobwhite quail and mallard ducks (Fletcher 1990a,b). In addition, 21 daily doses at both 1470 and 2150 mg a.i./kg bw, via gelatin capsule, caused no signs of toxicity in male or female bobwhite quail (Fletcher 1987). For this risk assessment, the 21-day exposure data from Fletcher (1987) will be used set an acute NOAEL of 2150 mg/kg bw for birds and this

value will be applied to all short-term (1-day) exposure assessments.

4.3.2.3. Terrestrial Invertebrates – As discussed in Section 4.1.2.3, tebufenozide mimics the invertebrate hormone 20-hydroxyecdysone and could cause adverse effects in a variety of terrestrial invertebrates. Notwithstanding this assertion, however, there are adequate field and field simulation studies clearly indicating that tebufenozide is much more toxic to *Lepidoptera* than to other insects.

Dose-response assessments for the effects of tebufenozide on terrestrial invertebrates could be based on either laboratory toxicity studies (Appendix 5) or field studies (Appendix 6). Most of the laboratory studies are on target rather than nontarget invertebrates and many involve exposures that are not readily applied to risk assessment. Studies that do involve both target and nontarget insects indicate that tebufenozide is more toxic to *Lepidoptera* (target species) than non-lepidopteran arthropods (Medina et al. 2002, 2003; Pietrantionio and Benedict 1999). In addition, tebufenozide appears to be less toxic to one nontarget species (lacewing) than diflubenzuron, another agent used to control the gypsy moth (Medina et al. 2002, 2003; Rumph et al. 1998).

The laboratory observations that non-lepidopteran arthropods are less sensitive to tebufenozide than *Lepidoptera* are supported by the field studies detailed in Appendix 6. A summary of the most relevant field studies is given in Table 4-3. In this table, efficacy studies summarized in Appendix 6 – i.e., those studies looking only at effects on target species, are omitted. Based on the study by Butler et al. (1997), both target and nontarget macrolepidoptera will be adversely affected at application rates as low as 0.03 lb/acre. Field studies at lower application rates have not been encountered and a NOAEL for nontarget macrolepidoptera cannot be identified. Similarly, a clear LOAEL for non-lepidopteran arthropods has not been identified. Mulder and Prescott (1999a) report a decrease in the numbers of beneficial arthropods on Day 3 after the application of tebufenozide at 0.125 lb a.i./acre but not at 0.24 lb a.i./acre. In addition, no effects on beneficial arthropods were seen at 0.125 lb/acre or 0.25 lb/acre on Day 5 to Day 15 after treatment.

For this risk assessment, the assumption is made that effects on sensitive nontarget *Lepidoptera* are likely to be comparable to those seen in target species. This assumption is based on the field study by Butler et al. (1997) in which a decrease in abundance in some lepidopteran species was noted after the application of Mimic 4F at rates of 0.03 and 0.06 lb a.i./acre. This may be a conservative assumption because, as noted by Butler et al. (1997), not all nontarget lepidopteran species were affected. Conversely, these investigators also noted that problems were encountered in the application of Mimic 4F, which resulted in poorer than expected efficacy. Thus, effects in nontarget lepidopteran species also may have been underestimated.

In the risk characterization, the minimum recommended application rate of 0.03 lb a.i./acre is taken as the exposure level that could be associated with adverse effects in some nontarget lepidopteran species. The true NOAEL in terms of application rate has not been defined for

nontarget lepidopteran species.

The potential for adverse effects on other nontarget insects is characterized quantitatively on the basis of the standard bioassay in the honey bee (Atkins. 1990; Chan 1995) in which no mortality was observed at doses of up to 233.98 µg a.i./bee or about 2500 mg/kg bw (see Section 4.1.2.3). As indicated in Table 4-2, this risk assessment also uses an application rate of 0.24 lb/acre as a functional NOEC for non-lepidopteran arthropods. This is based on the studies summarized in Table 4-3. As noted above, the application rate of 0.125 lb/acre from Mulder and Prescott (1999a) could be interpreted as a marginal LOEC. This interpretation would be grossly conservative because the effects seen at 0.125 lb/acre were transient and were not seen at 0.24 lb/acre.

Toxicity to soil invertebrates will be based on the standard toxicity bioassay in earthworms (Garvey 1992, discussed in Section 4.1.2.3) in which no effects were noted at soil concentrations of up to 1000 ppm (1000 mg/kg soil).

4.3.2.4. Terrestrial Plants and Microorganisms – As discussed in Sections 4.1.2.4. and 4.1.2.5., there is no reason to assume that tebufenozide will cause adverse effects in terrestrial plants or terrestrial microorganisms. Nonetheless, no standard toxicity studies have been encountered that could be used to quantify risk in either terrestrial plants or soil microorganisms. Consequently, no dose-response assessment for these groups can be proposed.

4.3.3. Aquatic Organisms.

4.3.3.1. Fish – The acute bioassays on fish summarized in Appendix 7 provide estimates of exposures which might be associated acute effects in fish but only two species have been tested. The most sensitive species is the bluegill sunfish with a 96-hour LC_{50} of 3.0 (2.2 to 4.0) mg/L with an NOEC of 0.39 mg/L (Graves and Smith 1992b). Rainbow trout appear to be somewhat less sensitive, with an LC_{50} value of 5.7 mg/L (4.7 to 6.5 mg/L) and an NOEC of 1.9 mg/L (Graves and Smith 1992c). For this risk assessment, the NOEC values of 0.39 mg/L and 1.9 mg/L are used to assess the consequences of short-term exposures for sensitive and tolerant species.

The assessment of the effects of tebufenozide that might be associated with chronic exposure to contaminated ambient water from the normal use and application of this product is based on the full life cycle study in fathead minnows by Rhodes and Leak (1996) supported by the egg and fry study by Bettancourt (1992).

In the egg and fry study (Bettancourt 1992), eggs were incubated at mean measured concentrations of 0, 0.084, 0.14, 0.22, 0.36, or 0.71 mg a.i./L by continuous exposure for 35 days. Based on a comparison to pooled controls (i.e., untreated and solvent treated animals with a combined survival of 94%), Bettancourt (1992) reports no effects on survival at any concentration level. The U.S. EPA (1999e), however, classified the 0.71 mg/L concentration as an effect level based on decreased survival (88%) relative to survival in the solvent control

(98%). The U.S. EPA analysis was challenged by Rohm and Haas (Surprenant 1994).

In the full life cycle study (Rhodes and Leak 1996), newly hatched eggs were exposed to mean measured concentrations of 0, 0.048, 0.090, 0.18, 0.35, or 0.72 mg a.i./L, again using both untreated and solvent (acetone) controls. The exposure was continued for 219 days which allowed for full development of the fish and reproduction. The most sensitive endpoint reported by Rhodes and Leak (1996) using pooled control data was survival with a LOAEL of 0.35 mg a.i./L and a NOAEL of 0.18 mg a.i./L. Again using solvent control rather than pooled control data, the U.S. EPA identified the most sensitive effect as decreased eggs/spawn and identified the LOAEL as 0.048 mg a.i./L, the lowest concentration tested. Because the U.S. EPA does not consider that this study identified a NOAEL, the U.S. EPA stated that the full life cycle study must be repeated (U.S. EPA 1999e). Again, the U.S. EPA analysis was contested by Rohm and Haas (Reinert et al. 1999).

The decision to pool or not pool control data is both statistical and judgmental, and the discussion provided by Reinert et al. (1999) is reasonably complete and objective. It is worth noting, nonetheless, that the statistical re-analysis presented by Reinert et al. (1999) does indicate that the dose-response relationship for eggs/spawn has p values of 0.077 or 0.058, depending on whether standard or weighted regression is used. Although these values may be classified as 'insignificant' using the standard cutoff p value of 0.05, the selection of this or any other p value is itself judgmental.

The statistical analyses of these studies are open to reasonable debate; however, the Forest Service attempts to maintain a consistency with the U.S. EPA unless there is a compelling reason to do otherwise. For this risk assessment, there appears to be no compelling reason to deviate from the U.S. EPA assessment. Notwithstanding the reasonable arguments put forth by Reinert et al. (1999), the effect of tebufenozide on eggs/spawn is at least marginally significant. Furthermore, the use of solvent control data leads to more conservative assessments of risk in both the egg and fry study as well as the full life cycle study. While this may be coincidental, the consistency between the two studies suggests that the differences could be related to some factor that is not fully understood at this time. Consequently, this risk assessment treats 0.048 mg/L, the lowest concentration tested in the full life cycle study, as a LOAEL for fish reproduction.

For this risk assessment, a LOAEL of 0.048 mg/L is adopted for chronic effects in fish. This interpretation of the study is identical to that of the U.S. EPA (1999e). The data are not sufficient to propose separate values for tolerant and sensitive species.

4.3.3.2. Aquatic Invertebrates – Although data on the effects of tebufenozide on aquatic invertebrates is limited to three species (i.e, daphnids, midge larvae and lobsters as summarized in Appendix 8), variability is apparent regarding the acute toxicity of tebufenozide to aquatic invertebrates. Based on the available bioassays, the most sensitive species is the midge (*Chironomus riparius*) with an acute LC_{50} of 0.3 mg/L and an NOEC of 0.12 mg/L (van der Kolk 1997). Daphnids appear to be much more tolerant, with an LC_{50} value of 3.8 mg/L and a

corresponding NOEC of 0.82 mg/L (Graves and Smith 1992a). The apparent high sensitivity of midge relative to *Daphnia* may be related to differences in the types of bioassays that are run on midges (sediment assays) compared to those run on *Daphnia* (water only without sediment). The highest reported NOEC in lobsters is 0.1 mg/L (Dionne 1998). Because the study on lobsters was conducted at very low concentrations and no effects were seen at any concentration, there is no basis for asserting that lobsters are sensitive species. For this risk assessment, the acute NOEC values of 0.12 mg/L and 0.82 mg/L are used to assess the consequences of short-term exposures for sensitive and tolerant species of aquatic invertebrates.

The midge is the most sensitive species for assessing the potential effects of chronic exposure. In the study by van der Kolk (1997), a concentration of 0.0053 mg/L caused a decrease in the larval emergence rate, and a concentration of 0.04 mg/L caused complete suppression of larval emergence. The NOAEL in this study is 0.0035 mg/L. Based on a standard 21-day reproductive study, *Daphnia magna* are substantially less sensitive with a reproductive NOEC of 0.029 mg/L and a corresponding LOEC of 0.059 mg/L (McNamara 1991). For this risk assessment, the longer-term NOEC values of 0.0035 mg/L and 0.029 mg/L are used to assess the consequences of longer-term exposures for sensitive and tolerant species of aquatic invertebrates.

4.3.3.2. Aquatic Plants – As with fish and invertebrates, the available studies (Section 4.3.3.4 and Appendix 8) suggest substantial differences in sensitivity among species of freshwater algae. For this risk assessment, risks to sensitive species are characterized using the lowest reported NOEC for algal growth of 0.077 mg/L in *Scenedesmus subspicatus* from the study by (Hoberg 1992a). An over eight-fold higher NOEC of 0.64 mg/L has been reported for *Selenastrum capricornutum* (Reinert 1993b) and this value will be used to characterize risks in tolerant algal species. Although these tests are conducted for relatively short periods of time (i.e., 96 to 120 hours), these NOEC values are applied to both acute and longer-term concentrations because of the short life-cycle of individual algal cells.

4.3.3.3. Aquatic Microorganisms – Other than the information on algae provided above, there are no data regarding the toxicity of tebufenozide to aquatic microorganisms. Accordingly, no dose-response assessment is possible for this group.

4.4. RISK CHARACTERIZATION

4.4.1. Overview

The use of tebufenozide to control the gypsy moth may result in adverse effects in nontarget *Lepidoptera* but there is little indication that other species will be impacted under normal conditions of use even at the highest application rate. Tebufenozide is an insecticide that is effective in controlling populations of lepidopteran pests. No data, however, are available on toxicity to nontarget *Lepidoptera*. For this risk assessment, the assumption is made that nontarget *Lepidoptera* may be as sensitive to tebufenozide as target *Lepidoptera*. Thus, adverse effects in nontarget *Lepidoptera* would be expected after applications that are effective for the control of lepidopteran pest species.

There is no indication that short term exposures to tebufenozide will cause direct adverse effects in any terrestrial vertebrates or non-lepidopteran invertebrates even at the upper range of plausible exposures as well as accidental exposures. Similarly, direct adverse effects from longer term exposures in birds and mammals appear to be unlikely under most conditions. In some extreme cases, exposures in some large mammals could exceed the NOEC but the exposures would be below levels that have been associated with frank signs of toxicity. Effects on birds due to a decrease in available prey – i.e., terrestrial invertebrates – may be plausible. Adverse effects in aquatic species are not expected under normal conditions of use. In the case of a large accidental spill into a relatively small body of water, however, adverse effects could be expected in aquatic vertebrates, invertebrates, and plants.

4.4.2. Terrestrial Organisms

4.4.2.1. Terrestrial Vertebrates – The risk characterization for terrestrial vertebrates is summarized in Worksheet G02 for the maximum application rate of 0.12 lb/acre. The risk characterization is based on the estimates of exposure summarized in Section 4.2.3 and the toxicity values for diflubenzuron derived in Section 4.3.2.1 and summarized in Table 4.2. For most exposure scenarios, hazard quotients are included for both single applications and two applications spaced three days apart. For those exposure scenarios that do not include both single and double applications, the exposures are based on two applications

None of the acute exposures result in hazard quotients that exceed the level of concern. The highest acute hazard quotient for any vertebrate is 0.04 – i.e., the consumption of contaminated fish by a fish-eating bird after an accidental spill – and this is below the level of concern by a factor of 20. Other more plausible exposure scenarios such as the consumption of contaminated vegetation and water are in the range of 0.000006 to 0.008, below the level of concern by factors of 125 to about 160,000.

Similarly, for longer term exposures, central and lower estimates of hazard quotients are substantially below a level of concern. The highest central estimate for any hazard quotient is 0.1 – i.e., below the level of concern by a factor of 10. At the upper ranges of exposure, however, the hazard quotient exceeds a level of concern for the consumption of contaminated vegetation on-site by a large mammal after either a single application (HQ=2) or two applications (HQ=4).

As noted in the dose response assessment for mammals, the hazard quotients for mammals are based on a NOAEL of 1.8 mg/kg/day from the study by Richards (1992a) in which the corresponding LOAEL – based on toxic effects in the blood – of 20 mg/kg/day. Thus, a hazard quotient of 11 [20 mg/kg/day ÷ 1.8 mg/kg/day] would suggest a high likelihood of adverse effects in blood. The estimated hazard quotients of 2 to 4 are below this level where adverse effects would be expected but some changes in blood could occur although the toxicologic significance of these effects would most likely be marginal because the 20 mg/kg/day dose group in the study by Richards (1992a) did not display any overt signs of toxicity. Another factor to consider in interpreting these risk quotients is the proportion of the animal's diet that is contaminated. The risk quotients for the consumption of contaminated vegetation that exceed the level of concern are all based on the assumption that 100% of the animal's diet is contaminated. In other words, the animal consumes only vegetation that has been directly sprayed with tebufenozide. Thus, the potential impact of canopy interception is not considered.

As discussed in Section 4.1.2.2 and detailed further in Appendix 6, the field study by Holmes (1998) noted suggestive effects on reproductive performance in Tennessee warblers – i.e., a decrease in the average number of eggs per nest and percent of eggs hatching. In addition, female warblers evidenced a decrease in brooding time and increase in foraging times, suggesting a decrease in prey availability. While the effects were not statistically significant, this study suggests that some birds may be impacted through a decrease in available prey secondary to the effects of tebufenozide on terrestrial invertebrates, as discussed further in Section 4.4.2.2.

The verbal interpretation of these risk quotients is thus somewhat uncertain. There is no indication that short term exposures to tebufenozide will cause adverse effects in any terrestrial vertebrates even at the upper range of plausible exposures as well as accidental exposures. Similarly, adverse effects from longer terms exposures in birds and mammals appears to be unlikely under most conditions. In some extreme cases, exposures in some large mammals could exceed the NOEC but the exposures would be below the known LOEC.

4.4.2.2. Terrestrial Invertebrates – Tebufenozide is an insecticide that is effective in controlling populations of lepidopteran pests. No data, however, are available on toxicity to nontarget *Lepidoptera*. For this risk assessment, the assumption is made that nontarget *Lepidoptera* may be as sensitive to tebufenozide as target *Lepidoptera*. Thus, applications of 0.03 lb/acre are considered a LOEC based on the studies summarized in Table 4-3. As noted in Section 4.3.2.3, a NOEC for target and nontarget *Lepidoptera* cannot be identified. The USDA may use application rates as low as 0.015 lb/acre and these applications are presumably effective in the control of the gypsy moth. Under the assumption that nontarget *Lepidoptera* are as sensitive to tebufenozide as target species, adverse effects in nontarget *Lepidoptera* would be expected.

Adverse effects in other insect species do not appear to be likely based on either the standard toxicity study in bees or the available field studies. As indicated in Worksheet G01, the hazard quotient for the direct spray of a bee is 0.08 at the maximum application rate of 0.12 lb/acre.

Based on field studies, application rates of up to 0.24 lb/acre appear to have no adverse effect on beneficial arthropods. Using application rates, the highest hazard quotient would be 0.5 [0.12 lb/acre ÷ 0.24 lb/acre]. Because effects on beneficial arthropods have not be examined at higher applications rates, the true NOEC for beneficial arthropods may be higher and perhaps substantially higher than 0.24 lb/acre. Consequently, the hazard quotient of 0.5 based on application rates is not inconsistent with the hazard quotient of 0.08 based on the honey bee toxicity bioassay.

Toxicity data are also available on earthworms in which no effects were noted at soil concentrations of up to 1000 ppm (1000 mg/kg soil) (Section 4.3.2.3). As noted in Table 4-1, the peak concentration that would be expected in soil after two applications at a rate of 0.12 lb/acre is about 0.1 ppm, below the level of concern by a factor of 10,000.

Thus, while the available data on nontarget terrestrial invertebrates are limited, it seems reasonable to assert that effected on nontarget lepidopterans are plausible at application rates that are effective in the control of target lepidopterans such as the gypsy moth. There is no basis for asserting that effects on other nontarget arthropods or other terrestrial invertebrates are plausible.

4.4.2.3. Terrestrial Plants and Microorganisms – No quantitative risk assessment to terrestrial plants is made for tebufenozide. As discussed in Section 4.1.2.4, there are no data on the toxicity of this compound to either terrestrial plants or microorganisms. This lack of data, however, adds no substantial uncertainty to this risk assessment. Tebufenozide has been extensively tested in both the laboratory and field studies for efficacy in the protection of terrestrial plants from insect pests. If tebufenozide were toxic to plants at applications at or substantially above those used to control the gypsy moth, it is likely that reports of such phytotoxicity would be noted. No such reports have been encountered.

4.4.3. Aquatic Organisms

A summary of the risk quotients for aquatic organisms is presented in worksheet G03. Risk characterizations are presented for sensitive and tolerant species of aquatic organisms (vertebrates, invertebrates, and plants) for three exposure scenarios (an accidental spill, expected peak concentrations, and expected longer term concentrations of tebufenozide in water). The expected peak and longer term concentrations are summarized in Table 3-5 and discussed in Section 3.2.3.4.6. The concentrations associated with an accidental spill are calculated in Worksheet D05 and discussed in Section 3.2.3.4.1. The toxicity values used for each group of organisms are summarized in Table 4-2 and discussed in Section 4.3.

The risk characterizations for each group of aquatic organisms are essentially identical. Under normal conditions of use at the highest anticipated application rate, no effects are expected in any group of organisms: vertebrates, invertebrates, or plants. In the case of an accidental spill, however, adverse effects would be expected in each group of organisms.

4.4.3.1. Aquatic Vertebrates – Under normal conditions of use, the highest hazard quotient for

sensitive species of fish is 0.1 – the hazard quotient associated with expected peak concentrations in water at the maximum anticipated application rate. The upper range of longer term concentrations in water are below a level of concern by a factor of about 33 (HQ=0.03). In the case of an accidental spill, however, the central estimate and the upper range of the hazard quotients exceeds a level of concern for both sensitive and tolerant species. As discussed in 3.2.3.4.1, the accidental spill scenario is both extreme and arbitrary, involving the spill of a relatively large amount of chemical into a small body of water.

4.4.3.2. Aquatic Invertebrates – Based on expected concentrations of tebufenozide in water under normal conditions of use, the upper ranges of the hazard quotients for sensitive aquatic invertebrates are 0.3 for short term peak concentrations and 0.4 for longer term concentrations. While these hazard quotients are somewhat higher than the corresponding hazard quotients for aquatic vertebrates, they are below a level of concern. In the case of an accidental spill, the concentrations in water exceed the level of concern for both sensitive and tolerant species of aquatic invertebrates.

4.4.3.3. Aquatic Plants – The risk characterization for aquatic plants is based on bioassay data using algae. Because bioassay on algae are conducted only over relatively short periods of time – i.e., 96 to 120 hours – the toxicity values for both tolerant and sensitive species of algae are all essentially short term. As with both aquatic vertebrates and invertebrates, none of the expected concentrations in water exceed the level of concern for sensitive or tolerant species of algae even at the upper ranges of plausible exposures. Also as with aquatic vertebrates and invertebrates, the level of concern is exceed for both sensitive and tolerant species of algae in the case of an accidental spill.

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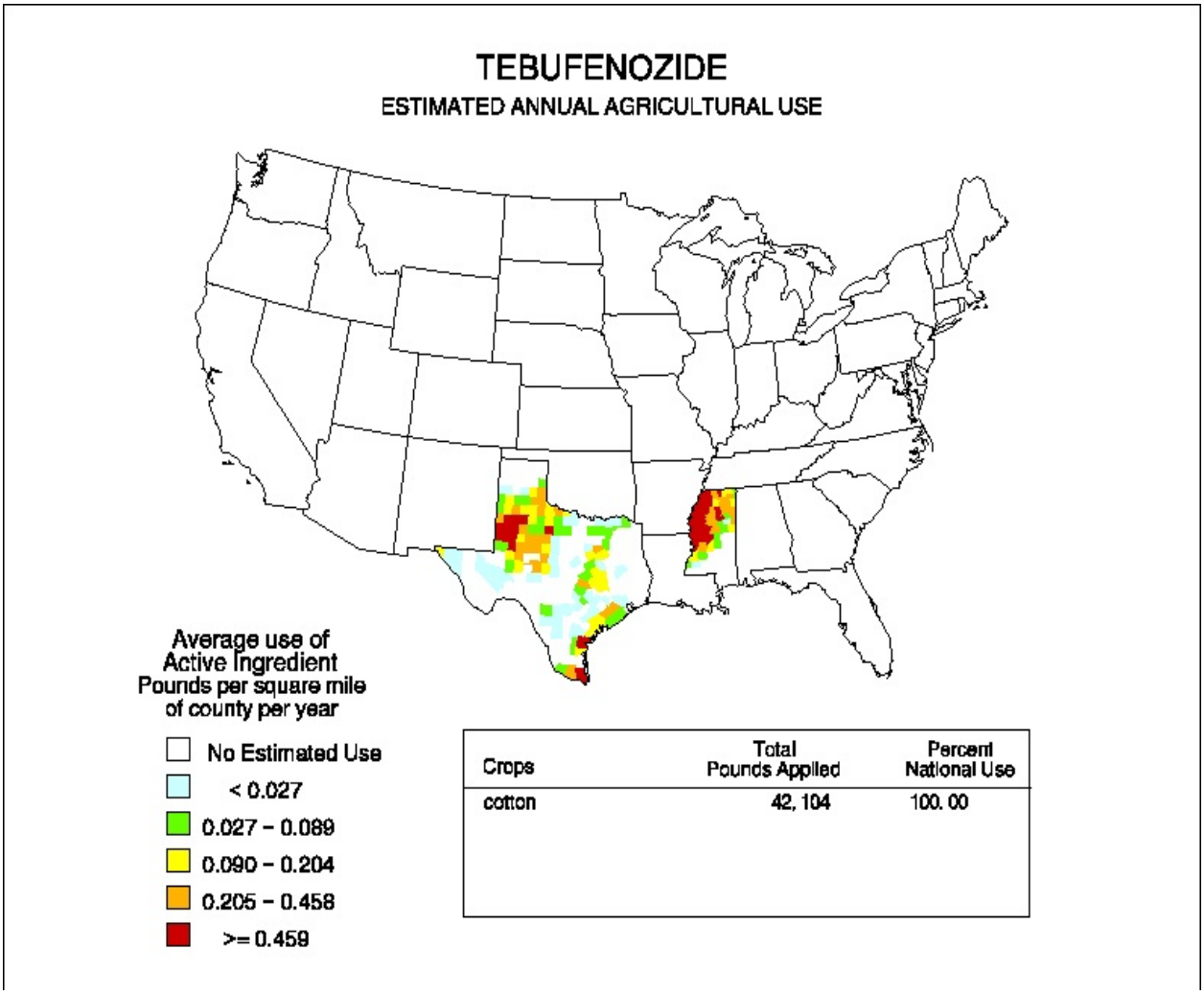

Figure 2-1: Agricultural Use of Tebufenozide on Cotton in 1992 (USGS 1998).

Table 2-1. Selected physical and chemical properties of tebufenozide with selected additional properties for the commercial formulation Mimic.

Appearance, ambient	Mimic: off-white, cream color liquid. (C&P Press 2004) Tebufenozide, technical: while solid (Kelly 1992)
Bioconcentration factor	151 in whole fish (Dong and Hawkins. 1993) 16 in edible tissue (Dong and Hawkins. 1993)
CAS number	112410-23-8 (C&P Press 2004; Kelly 1992)
Commercial formulations	Mimic 2LV; Confirm 2F
EPA Registration Number	707-237 (Patel 1998)
Foliar half-time (days)	2.8 to 13.3 days (Hawkins 1998) 11.3 to 14 days (Kaminski 1997) about 18.4 to 58.7 days (Sundaram et al. 1996a, Table 6, p. 725) about 20 days (white spruce) (Sundaram et al. 1996b,)
Foliar wash-off fraction	0.3 to 0.7 Sundaram et al. (1997b, Table 6, p. 514) 0.2 to 0.8 Sundaram (1994b)
log $K_{o/w}$	4.25 (Hawkins 1995) 4.25 (SRC 1999)[$K_{o/w}$ = 17,800]
Molecular weight	352.48 (Patel 1998)
pH	6.5-7.5 (C&P Press 2004)
Photolysis (days)	98[soil surface] (Hawkins 1995) 67[in aqueous solution] (Hawkins 1995)
Soil half-time (days)	99 to 101[aerobic] (Hawkins 1995) 66[aerobic] (Kaminski 1997)
Soil sorption, $K_{o/c}$	572 (Hawkins 1995)
Specific Gravity	Mimic: 1.0 (C&P Press 2004)
Synonyms	3,5-dimethyl-, 1-(1,1-dimethylethyl)-benzoic acid (C&P Press 2004) N-tert-butyl-N'-(4-ethylbenzoyl)-3,5-dimethylbenzol)hydrazide (Kaminski 1997) RH-5992 (Kelly 1992), Confirm
Vapor pressure	17 mm Hg @ 20°C/68°F (C&P Press 2004) 2×10^{-8} torr at 25°C (Kaminski 1997)
Volatility	60% (C&P Press 2004)
Water solubility (mg/L)	0.83 (Kaminski 1997)

Table 3-1: Chemical and site parameters used in GLEAMS modeling for tebufenozide.

Chemical Specific Parameters

Parameter	Clay	Loam	Sand	Comment/ Reference
Halftimes (days)				
Aquatic Sediment		179		U.S. EPA 1999e, p. 5
Foliar		13.4		Note 1
Soil	100	270	730	Note 2
Water		67		Note 3
Ko/c, mL/g		572		Note 4
K_d, mL/g	7.8	4.4	1.7	Note 5
Water Solubility, mg/L		0.83		Kaminski 1997
Foliar wash-off fraction		0.5		Note 6
Fraction applied to foliage		0.8		

Note 1 Geometric mean of range of values from Table 2-1: 3 to 60 days.

Note 2 The soil half time for sand is taken as 730 days, the value used by U.S. EPA (1999e) in PRZM/EXAMS modeling. For clay, a soil halftime of 100 days is used (Hawkins 1995). As an intermediate value, the geometric mean of this range is used for loam.

Note 3 Photolysis halftime used by U.S. EPA 1999e from study by Hawkins 1995.

Note 4 This is taken from Hawkins (1995) and is identical to the value used by U.S. EPA (1999e) in the PRZM/EXAMS modeling

Note 5 Taken from U.S. EPA (1999e), Table 1, p. 6.

Note 6 Sundaram et al. (1997) have reported wash-off fractions 30% to 70% (Table 6, p. 514). Somewhat wider ranges, 20% to 80%, have been reported by Sundaram (1994b). For the GLEAMS modeling, a central value of 50% is used.

Site Parameters
(see SERA 2004b for details)

Pond	1 hectare pond, 2 meters deep, with a 0.01 sediment fraction. 10 hectare square field (1093' by 1093') with a root zone of 12 inches.
Stream	Base flow rate of 710,000 L/day with a flow velocity of 0.08 m/second or 6912 meters/day. Stream width of 2 meters (about 6.6 feet'). 10 hectare square field (1093' by 1093') with a root zone of 12 inches.

Table 3-2: Summary of modeled concentrations of tebufenozide in streams (all units are µg/L or ppb)

Annual Rainfall (inches)	Rainfall per Event (inches)[1]	Clay		Loam		Sand	
		Average	Maximum	Average	Maximum	Average	Maximum
		Concentration per lb/acre applied (from GLEAMS)					
5	0.14	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
10	0.28	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
15	0.42	0.69713	19.95600	0.00878	0.29002	1.90923	52.54274
20	0.56	1.68973	54.33504	0.06773	1.43491	5.30526	101.05556
25	0.69	2.55255	91.00476	0.16814	3.12871	7.05234	111.28758
50	1.39	4.09339	219.00699	0.77041	11.44738	6.85127	93.61309
100	2.78	3.52070	317.12471	1.34698	30.36614	4.42689	88.43373
150	4.17	2.70849	334.75298	1.35142	45.96028	3.16969	88.64864
200	5.56	2.16187	320.13751	1.24326	55.46092	2.43988	87.51616
250	6.94	1.78771	287.69153	1.12607	60.75455	1.97609	84.88519
	Application rate:	**0.12**	lbs/acre				
		Concentration at above application rate					
5	0.14	0	0	0	0	0	0
10	0.28	0	0	0	0	0	0
15	0.42	0.083656	2.39472	0.00105	0.034802	0.2291076	6.3051288
20	0.56	0.2027676	6.5202048	0.00813	0.1721892	0.6366312	12.126667
25	0.69	0.306306	10.920571	0.020177	0.3754452	0.8462808	13.35451
50	1.39	0.4912068	26.280839	0.092449	1.3736856	0.8221524	11.233571
100	2.78	0.422484	38.054965	0.1616376	3.6439368	0.5312268	10.612048
150	4.17	0.3250188	40.170358	0.1621704	5.5152336	0.3803628	10.637837
200	5.56	0.2594244	38.416501	0.1491912	6.6553104	0.2927856	10.501939
250	6.94	0.2145252	34.522984	0.1351284	7.290546	0.2371308	10.186223

[1] Rain is assumed to occur at the same rate every 10th day – i.e., 36 rainfall events per year.

Table 3-3: Summary of modeled concentrations of tebufenozide in ponds (all units are µg/L or ppb)

Annual Rainfall (inches)	Rainfall per Event (inches)[1]	Clay		Loam		Sand	
		Average	Maximum	Average	Maximum	Average	Maximum
		Concentration per lb/acre applied (from GLEAMS)					
5	0.14	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
10	0.28	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
15	0.42	1.62583	3.41905	0.01831	0.04465	4.17974	8.26554
20	0.56	3.01439	9.47016	0.10599	0.18515	8.82060	13.48834
25	0.69	4.18885	16.64130	0.23102	0.36543	10.95654	15.44082
50	1.39	7.25113	51.67100	0.93903	1.28274	11.29006	26.68412
100	2.78	8.47509	103.59184	2.06369	6.79246	8.75309	39.33410
150	4.17	7.95210	134.03042	2.47999	16.52847	7.16252	45.03134
200	5.56	7.23386	157.87981	2.59791	25.60810	6.09099	47.50864
250	6.94	6.58435	168.88316	2.59975	32.69145	5.32904	48.43668
	Application rate:	**0.12**	lbs/acre				
		Concentration at above application rate					
5	0.14	0	0	0	0	0	0
10	0.28	0	0	0	0	0	0
15	0.42	0.1950996	0.410286	0.0022	0.00536	0.5015688	0.9918648
20	0.56	0.3617268	1.1364192	0.012719	0.022218	1.058472	1.6186008
25	0.69	0.502662	1.996956	0.027722	0.043852	1.3147848	1.8528984
50	1.39	0.8701356	6.20052	0.1126836	0.1539288	1.3548072	3.2020944
100	2.78	1.0170108	12.431021	0.2476428	0.8150952	1.0503708	4.720092
150	4.17	0.954252	16.08365	0.2975988	1.9834164	0.8595024	5.4037608
200	5.56	0.8680632	18.945577	0.3117492	3.072972	0.7309188	5.7010368
250	6.94	0.790122	20.265979	0.31197	3.922974	0.6394848	5.8124016

[1] Rain is assumed to occur at the same rate every 10th day – i.e., 36 rainfall events per year.

Table 3-4: Estimated Environmental Concentrations (µg/L or ppb) of tebufenozide in surface and groundwater at two applications of 0.12 lb a.i./acre (0.134 kg/ha), three days apart.

Scenario	Peak	Long-Term Average
	MODELING FOR THIS RISK ASSESSMENT	
Direct Spray of Pond (Worksheet 04b)	6.73	N/A
Pond, drift at 100 feet (Worksheet 04b)	0.13	N/A
GLEAMS, Stream	10 (0.03 to 40)	0.3 (0.001 to 0.8)
GLEAMS, Pond	5 (0.005 to 20)	0.5 (0.002 to 1.4)
GENEEC Version 2, Pond	8.21	1.5 [90 day value of 6.01 x 90/360]
Sci-Grow 2.3, groundwater	0.093	
	OTHER MODELING	
U.S. EPA/OPP 1999e.PRZM/EXAMS modeling of application to apples, Pond	8.7 ppb at 6x0.31 lb/ac	5.4 ppb at 6x0.31 lb/ac
U.S. EPA/OPP 1999e.PRZM/EXAMS modeling of application to cotton, Pond	17 ppb at 4x0.25 lb/ac	8.2 ppb at 4x0.25 lb/ac
	MONITORING STUDIES	
Sundarum et al. 1996a	At an application rate of 2x0.070 kg/ha (0.062 lb/acre) with a 4 day interval. Peak stream concentrations of 1.32 ppb and peak pond concentrations of 5.31 ppb. Concentrations were below the limit of quantization limit of 0.04 µg/L by day 24 after application. Pond=300,000 liters in volume, 500 m^2 surface area, 0.6 m deep. Stream width=2m, depth=20 cm, 7 m/min flow.	

Table 3-5: Concentrations of tebufenozide in surface water used in this risk assessment (see Section 3.2.3.4.6 for discussion).

At application rate:	**0.12**	**lb/acre, 2 applications, 3 days apart**	
		Peak Concentration (ppb or µg/L)	Longer Term Concentration (ppb or µg/L)
	Central	10	0.5
	Lower	0.005	0.002
	Upper	40	1.4
Water contamination rate [1]		**mg/L per lb/acre applied, 2 applications, 3 days apart**	
		Peak Concentration (mg/L per lb/acre)	Longer Term Concentration (mg/L per lb/acre)
	Central	8.33e-02	4.17e-03
	Lower	4.17e-05	1.67e-05
	Upper	3.33e-01	1.17e-02

[1] Water contamination rates – concentrations in units of mg/L expected at an application rate of 1 lb/acre. These values are entered into Worksheet B06a for diflubenzuron. This rate is adjusted to the program application rate in all worksheets involving exposure to contaminated water.

Table 4-1: Summary of modeled concentrations of tebufenozide in soil (all units are mg/kg or ppm), two applications spaced three days apart.

Annual Rainfall (inches)	Rainfall per Event (inches)[1]	Clay		Loam		Sand	
		Average	Maximum	Average	Maximum	Average	Maximum
		Concentration per lb/acre applied (from GLEAMS)					
5	0.14	0.14894	0.33141	0.29680	0.49427	0.50678	0.84666
10	0.28	0.15592	0.33655	0.31226	0.51438	0.51705	0.86709
15	0.42	0.14905	0.33070	0.29440	0.48949	0.48422	0.79343
20	0.56	0.14349	0.32703	0.29249	0.48803	0.43053	0.67757
25	0.69	0.13746	0.32353	0.29102	0.48656	0.37176	0.57116
50	1.39	0.10849	0.30803	0.27746	0.46370	0.20593	0.34765
100	2.78	0.06705	0.27677	0.22935	0.39646	0.10536	0.28079
150	4.17	0.04360	0.24522	0.19143	0.35247	0.07083	0.27603
200	5.56	0.03094	0.21427	0.16493	0.32387	0.05381	0.27361
250	6.94	0.02313	0.18274	0.14567	0.30341	0.04358	0.27084
	Application rate:	**0.12**	lbs/acre				
		Concentration at above application rate					
5	0.14	0.017873	0.039769	0.035616	0.059312	0.060814	0.1015992
10	0.28	0.01871	0.040386	0.037471	0.061726	0.062046	0.1040508
15	0.42	0.017886	0.039684	0.035328	0.058739	0.058106	0.095212
20	0.56	0.017219	0.039244	0.035099	0.058564	0.051664	0.081308
25	0.69	0.016495	0.038824	0.034922	0.058387	0.044611	0.068539
50	1.39	0.013019	0.036964	0.033295	0.055644	0.024712	0.041718
100	2.78	0.00805	0.033212	0.027522	0.047575	0.012643	0.033695
150	4.17	0.00523	0.029426	0.022972	0.042296	0.0085	0.033124
200	5.56	0.00371	0.025712	0.019792	0.038864	0.00646	0.032833
250	6.94	0.00278	0.021929	0.01748	0.036409	0.00523	0.032501

[1] Rain is assumed to occur at the same rate every 10th day – i.e., 36 rainfall events per year.

Table 4-2: Summary of tebufenozide toxicity values used in ecological risk assessment

Organism	Endpoint	Toxicity Value	Reference, Species
Mammals (Rats and Rabbits)	Acute NOAEL, reproduction	1000 mg/kg	Swenson and Solomon 1992 (rabbits) Hoberman 1991 (rats)
	Chronic NOAEL, toxicity	1.8 mg/kg/day	Richards 1992a
Birds (Bobwhite Quail)	Acute NOAEL	2150 mg/kg	Fletcher 1987
	Chronic NOAEL	15 mg/kg/day	Beavers et al. 1993b [1]
Terrestrial Invertebrates			
Honey bee	NOEC	2500 mg/kg	Atkins (1990) and Chan (1995)
Tolerant Insect Species	NOEC	0.24 lb a..i. /acre	Mulder and Prescott 1999a,b
Sensitive Lepidoptera	LOEC	0.03 lb a..i./acre	Butler et al. (1997)
Earthworm	NOEC	1000 mg/kg soil	Garvey (1992)
Fish Acute			
Sensitive (Bluegills)	NOEC	0.39 mg/L	Graves and Smith (1992b)
Tolerant (Trout)	NOEC	1.9 mg/L	Graves and Smith (1992c)
Fish Chronic			
Sensitive/Tolerant (Fathead Minnows)	LOEC, reproduction	0.048 mg/L	Rhodes and Leak (1996) as interpreted by U.S. EPA (1999e) [3]
Aquatic Invertebrates, Acute			
Sensitive (Midge larvae)	NOEC	0.12 mg/L	van der Kolk (1997)
Tolerant (Daphnids)	NOEC	0.82 mg/L	Graves and Smith (1992a)
Aquatic Invertebrates, Chronic			
Sensitive (Midge larvae)	NOEC, reproduction	0.0035 mg/L	van der Kolk (1997)
Tolerant (Daphnids)	NOEC, reproduction	0.029 mg/L	McNamara (1991)
Aquatic Plants			
Sensitive *(Scenedesmus subspicatus*)	NOEC for growth	0.077 mg/L	Hoberg (1992a)
Tolerant (*Selenastrum capricornutum*)	NOEC for growth	0.64 mg/L	Reinert (1993b)

[1] Other studies are available indicating higher NOAELs. See 4.3.2.2 for discussion.
[2] Other studies are available indicating no effects on tolerant invertebrates at application rates up to 0.25 lb/acre. See Table 4-3 and Section 4.3.2.3 for discussion.
[3] See Section 4.3.3.1 for a discussion of interpretation of studies.

Table 4-3: Summary of field studies on the effects of tebufenozide on terrestrial invertebrates [1]

Range of Application Rates (lb a.i./acre)	Species	
	No Adverse Effects	**Adverse Effects**
0.03 - <0.06	abundance of non-target arthropods other than macrolepidoptera (0.03 – Butler et al. 1997)	abundance of various macrolepidoptera (0.03 – Butler et al. 1997)
0.06 - < 0.12	abundance of non-target arthropods other than macrolepidoptera (0.06 – Butler et al. 1997)	abundance of various macrolepidoptera (0.06 – Butler et al. 1997) spruce budworm (0.06 – Cadogan et al. 1997)
0.12 - < 0.24	spiders, lacewings, and predatory mites (0.23 – Gurr et al. 1999) Mexican rice borer (0.12 and 0.18 – Legaspi et al. 1999) various beneficial arthropods* (0.125 – Mulder and Prescott 1999a)	spruce budworm (0.12 – Cadogan et al. 1997) various lepidopteran pests (0.23 – Gurr et al. 1999) beet armyworm (0.125 – Mulder and Prescott 1999a)
0.24	various beneficial arthropods (0.24 – Mulder and Prescott 1999a) beneficial arthropods (0.24 – Mulder and Prescott 1999b)	beet armyworm (0.24 – Mulder and Prescott 1999a) potato leafhopper (0.25 – Mulder and Prescott 1999b)

[1] Studies summarized in Appendix 6 with some efficacy studies omitted. The application rate in lb/acre and citation is given in parenthesis following the species or group. See text for discussion. A single asterisk (*) indicates transient or equivocal effects.

LIST OF APPENDICES

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Appendix 1: Estimates of dermal absorption rates for tebufenozide

Table A1-1: Estimate of first-order absorption rate (k_a in hours^{-1}) and 95% confidence intervals.

Model parameters	ID	Value	
Coefficient for $k_{o/w}$	C_KOW	0.233255	
Coefficient for MW	C_MW	0.005657	
Model Constant	C	1.49615	
Number of data points	DP	29	
Degrees of Freedom (d.f.)	DF	26	
Critical value of $t_{0.025}$ with 26 d.f.[a]	CRIT	2.056	
Standard error of the estimate	SEE	16.1125	
Mean square error or model variance	MDLV	0.619712	
Standard deviation of model (s)	MSD	0.787218	$MDLV^{0.5}$
X′X, cross products matrix	0.307537	-0.00103089	0.00822769
	-0.00103089	0.000004377	-0.0000944359
	0.0082	-0.0000944359	0.0085286

[a] Mendenhall and Scheaffer, 1973, Appendix 3, 4, p. A31.

Central (maximum likelihood) estimate:

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

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

$$\log_{10} k_a \pm \boldsymbol{t}_{0.025} \times \boldsymbol{s} \times (\boldsymbol{a}'\boldsymbol{X}'\boldsymbol{X}\,\boldsymbol{a})^{0.5}$$

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

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

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

Worksheet A07a (continued)
Details of calculating a′X′X a

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

Letting

$$\mathbf{a} = \{a\_1, a\_2, a\_3\}$$

and

$$(\mathbf{X'X})^{-1} = \{ \{b\_1, b\_2, b\_3\}, \{c\_1, c\_2, c\_3\}, \{d\_1, d\_2, d\_3\} \},$$

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

Term 1: {a_1 ×([a_1×b_1] + [a_2×c_1] + [a_3×d_1])} +
Term 2: {a_2 ×([a_1×b_2] + [a_2×c_2] + [a_3×d_2])} +
Term 3: {a_3 ×([a_1×b_3] + [a_2×c_3] + [a_3×d_3])}.

Table A1-2: Calculation of first-order dermal absorption rate (k_a) for tebufenozide.							
Parameters	Value	Units	Reference				
Molecular weight	352.48	g/mole	Table 2-1				
$K_{o/w}$ at pH 7	17,800	unitless	Table 2-1				
$\log_{10} K_{o/w}$	4.25						
Column vector ***a*** for calculating confidence intervals (see Worksheet A07a for definitions.)							
a_1	1						
a_2	352.48						
a_3	4.25						
Calculation of $\mathbf{a'} \cdot (\mathbf{X'X})^{-1} \cdot \mathbf{a}$ - see Worksheet A07a for details of calculation.							
Term 1	-0.0209811072						
Term 2	0.0389710295						
Term 3	0.0475467644						
$\mathbf{a'} \cdot (\mathbf{X'X})^{-1} \cdot \mathbf{a}$	0.0655	calculation verified in Mathematica 3.0.1.1					
$\log_{10} k_a = 0.233255 \log_{10}(k_{o/w}) - 0.005657\ MW - 1.49615$			Worksheet A07a				
$\log_{10}$ of first order absorption rate (k_a)							
Central estimate	-2.49869764236	±	$t_{0.025}$	×	s	×	$(\mathbf{a'} \cdot (\mathbf{X'X})^{-1} \cdot \mathbf{a})^{0.5}$
Lower limit	-2.91292499777	-	2.0560	×	0.787218	×	0.2559296778
Upper limit	-2.08447028695	+	2.0560	×	0.787218	×	0.2559296778
First order absorption rates (i.e., antilog or 10^x of above values).							
Central estimate	0.003171775	$hours^{-1}$					
Lower limit	0.001222011	$hours^{-1}$					
Upper limit	0.008232462	$hours^{-1}$					

Table A1-3: Estimate of dermal permeability (K_p in cm/hr) and 95% confidence intervals.

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

[a]Mendenhall and Scheaffer, 1973, Appendix 3, Table 4, p. A31.

NOTE: The data for this analysis are taken from U.S. EPA (1992), Dermal Exposure Assessment: Principles and Applications, EPA/600/8-91/011B, Table 5-4, pp. 5-15 through 5-19. The U.S. EPA report does not provide sufficient information for the calculation of confidence intervals. The synopsis of the above analysis was conducted in STATGRAPHICS Plus for Windows, Version 3.1 (Manugistics, 1995) as well as Mathematica, Version 3.0.1.1 (Wolfram Research, 1997). Although not explicitly stated in the U.S. EPA report, 3 of the 93 data points are censored from the analysis because they are statistical outliers: [Hydrocortisone-21-yl]-hemipimelate, n-nonanol, and n-propanol. The model parameters reported above are consistent with those reported by U.S. EPA but are carried out to a greater number of decimal places to reduce rounding errors when calculating the confidence intervals. See notes to Worksheet A07a for details of calculating maximum likelihood estimates and confidence intervals.

Table A1-4**:** Calculation of dermal permeability rate (K_p) in cm/hour for tebufenozide.								
Parameters	Value	Units					Reference	
Molecular weight	352.48	g/mole						
$K_{o/w}$ at pH 7	17800	unitless						
$\log_{10} K_{o/w}$	4.25							
Column vector ***a*** for calculating confidence intervals (see Worksheet A07a for definitions.)								
a_1	1							
a_2	352.48							
a_3	4.25							
Calculation of $\mathbf{a}' \cdot (\mathbf{X}'\mathbf{X})^{-1} \cdot \mathbf{a}$ - see Worksheet A07b for details of calculation.								
Term 1	-0.0220577884							
Term 2	0.007751756							
Term 3	0.0564889197							
$\mathbf{a}' \cdot (\mathbf{X}'\mathbf{X})^{-1} \cdot \mathbf{a}$	0.0422	calculation verified in Mathematica 3.0.1.1						
$\log_{10} k_p = 0.706648 \log_{10}(k_{o/w}) - 0.006151\ MW - 2.72576$							Worksheet A07b	
$\log_{10}$ of dermal permeability								
Central estimate	-1.89061048	±	$t_{0.025}$	×	s	×	$\mathbf{a}' \cdot (\mathbf{X}'\mathbf{X})^{-1} \cdot \mathbf{a}^{0.5}$	
Lower limit	-2.18337858572	-	1.9600	×	0.727129	×	0.2054263858	
Upper limit	-1.59784237428	+	1.9600	×	0.727129	×	0.2054263858	
Dermal permeability								
Central estimate	0.0128644	cm/hour						
Lower limit	0.0065557	cm/hour						
Upper limit	0.025244	cm/hour						

Table A1-5: Summary of chemical specific dermal absorption values used for tebufenozide dermal absorption.				
Description	Code	Value	Units	Reference/Source
First-order absorption rates (k_a)				
Central estimate	AbsC	0.0032	$hour^{-1}$	Table A1-2, values rounded to two significant figures
Lower limit	AbsL	0.0012	$hour^{-1}$	
Upper limit	AbsU	0.0082	$hour^{-1}$	
Zero-order absorption (K_p)				
Central estimate	KpC	0.013	cm/hour	Table A1-4, values rounded to two significant figures
Lower limit	KpL	0.0066	cm/hour	
Upper limit	KpU	0.025	cm/hour	

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference
ACUTE			
Mice (NOS)	>5.0 g/kg technical, single oral dose (NOS)	No treatment related mortalities or signs of toxicity at limit dose of 5.0 g/kg LD_{50}>5.0 g/kg	Hazleton and Quinn 1995b MRID 43781708
Rats, Crl:CD, 29 to 34-days old, weighing 73-101 g, 10 males and 10 females per dose group	0, 500, 1000, or 2000 mg/kg bw by gavage (single dose)	No treatment-related mortalities, clinical signs of toxicity, or effects on body weight at any dose level; no neurotoxic or neuropathological effects at any dose level. NOEL >2000 mg/kg bw (highest dose tested)	Swenson et al. 1994 MRID 43781706
Rats, CD, adults, 6 males and 6 females	single gavage dose of 5.0 g/kg bw Mimic® 240 LV	No mortalities, body weight effects, or clinical signs of toxicity. Acute oral LD_{50} >5.0 g/kg bw or 5000 mg/kg This study reveals the components of Mimic formulation. This information cannot be disclosed in this document.	Parno and Gingrich. 1994b MRID 44727702
Rats (NOS)	>5.0 g/kg technical, single oral dose (NOS)	"practically non-toxic;" no treatment-related mortalities or signs of toxicity at the limit dose of 5.0 g/kg LD_{50}>5.0 g/kg	Hazleton and Quinn 1995b MRID 43781708 (This appears to be a summary of Parno and Gingrich 1994b, detailed above)
SUBCHRONIC			
Dogs, 4 males and 4 females per dose group (NOS)	0, 150, 600, 2400, or 9600 ppm ai in diet for 2 weeks	No effects on body weight or food consumption and no clinical or gross observations of toxicity. No effects at 150 ppm ai (5.1 mg/kg bw/day) At ≥600 ppm ai, increased spleen weight was noted; at ≥2400 ppm ai, increased spleen-to-body weight ratio was noted; at 9600 ppm ai, additional adverse effects included decreased RBC, hemoglobin, and hematocrit values.	Hazleton and Quinn 1995b MRID 43781708 (Hazard evaluation/data summary)

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference
Dogs, one male and one female per dose group (NOS)	limit dose of 30,000 ppm ai (1000 mg/kg bw/day) in diet for 2 weeks	decrease in food consumption during week 1 but not week 2 (both sexes); decreased body weight (male), hematological effects (both sexes) included decreased RBC, hemoglobin, and hematocrit values, increased methemoglobin (females), reticulocytes, Heinz bodies, platelets and white blood cells. Treatment-related effects included increased bilirubin and other changes in serum chemistry (NOS) and increased spleen weights above the upper limit expected for this species. Limit dose of 30,000 ppm was considered too high to be used in 13-week study.	Hazleton and Quinn 1995b MRID 43781708 (Hazard evaluation/data summary)
Dogs, males, 4 per dose group (NOS)	0 or 1500 ppm ai technical for 6 weeks, followed by control diet (0 ppm) for additional 4 weeks; hematological parameters were measured in controls and treated dogs prior to treatment, at 6 weeks, at 8 weeks, and at 10 weeks	Study designed to examine reversibility of hematological effects after exposure to RH-5992 technical. After 6 weeks, hematological effects in treated dogs included decreases in RBC, hemoglobin, and hematocrit values; increases in methemoglobin, mean corpuscular volume, reticulocytes, and platelets. Complete recovery (i.e., effects on hemopoietic system returned to control values) by the end of the 2- or 4-week recovery period.	Hazleton and Quinn 1995b MRID 43781708 (Hazard evaluation/data summary)
Dogs, beagles, purebred, ~8-months old, 4 males and 4 females per dose group	oral administration by admixture of 0, 50, 500, or 5000 ppm (active ingredient) for 90 days; group mean compound consumption in mg/kg/day for 13 weeks was: 2.09, 20.13, or 202.42 mg/kg/day (FEMALES) and 2.05, 21.42, or 201.82 mg/kg/day (MALES)	Dietary concentrations of 500 or 5000 ppm had a direct effect on red blood cells, leading to low grade hemolytic anemia. NOEL = 50 ppm No clinical signs of toxicity were attributed to treatment; high dose males gained slightly less weight than controls but the difference was not statistically significant; high dose males and females ate slightly less food than controls but the difference was not statistically significant; treatment had no effect on food conversion efficiency; and no ocular lesions resulted from treatment.	Clay 1992 MRID 42436223

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference
Additional Observations from Clay 1992 MRID 42436223: **Hematology**: there were several statistically significant effects on hematological parameters (e.g., red blood cell count, mean cell volume, reticulocyte counts, methemoglobin, incidence of Heinz bodies, and platelet counts) in males and females exposed to 500 or 5000 ppm. The presence of Heinz bodies is considered to represent a direct effect on the RBC and led to increased destruction of RBC in liver and spleen. **Urinalysis**: urine of treated males was darker than urine of controls in week 13; three high dose males had bilirubin present in their urine (consistent with destruction of red blood cells). **Organ weights**: in high dose males, mean absolute spleen weight was 30% greater than that of controls ($p \leq 0.05$) and relative spleen weight was 44% greater ($p \leq 0.01$); in females there was a significant dose response in relative spleen weight ($p \leq 0.05$); no statistically significant differences in relative liver weight among treated dogs; in high dose females, there was a statistically significant dose response with respect to increased liver weight. Various treatment-related effects indicative of low grade hemolytic anemia were observed in the liver (increased incidence of pigment in the Kupffer cells), spleen (increased hemopoiesis and increased sinusoidal engorement) and bone marrow (hyperplasia) of males and female exposed to 500 or 5000 ppm.			
Mice, males, 8 per dose group (NOS)	0, 60, 200, 600, 2000 or 6000 ppm ai technical in diet for 2 weeks	No effects at ≤600 ppm; increased liver-to-body weight ratio at 2000 or 6000 ppm; increased liver weight at 6000 ppm (~1000 mg/kg bw/day); no adverse effects on survival, clinical chemistry, body weight or food consumption.	Hazleton and Quinn 1995b MRID 43781708 (Hazard evaluation/data summary)
Mice, Crl:CD-1, ~4-weeks old, 10 males and 20 females per dose group	0, 20, 200, 2000 or 20,000 ppm in the diet for 13 weeks	No mortality; no treatment related clinical, cageside, or ophthalmoscopic observations. **Body weight**: significantly decreased mean body weight values at weeks 0-13 in males at 200 or 2000 ppm and at weeks 0-4 and 0-13 in males at 20,000 ppm; no statistically significant differences in mean food consumption values among all dose groups.	Osheroff 1991a MRID 42436221

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference
Additional Notes on Osheroff 1991a MRID 42436221 **Hematology**: significant increases in reticulocyte and absolute reticulocyte counts (males and females at 2000 or 20,000 ppm), mean cell volume (males at 2000 or 20,000 ppm), mean cell hemoglobin (males and females at 2000 or 20,000 ppm), mean cell hemoglobin concentration (males at 2000 and males and females at 20,000 ppm), white blood cell count, corrected white blood cell count, and lymphocyte counts (females at 2000 ppm and males and females at 20,000 ppm), heinz bodies (males at 2000 ppm and males and females at 20,000 ppm), and segmented neutrophils (males at 2000 ppm and males and females at 20,000 ppm). Decreased erythrocyte counts in males and female at 2000 or 20,000 ppm (significant only in males), decreased myeloid/erythroid ratios in males and female at 2000 or 20,000 ppm (significant only in females), significant increases in methemoglobin values in males and females at 2000 or 20,000 ppm, significant increased mean alkaline phosphatase and potassium values in males at 2000 or 20,000 ppm and significantly increased mean total protein and calcium values in males at 20,000 ppm. **Organ weights**: significant decrease in mean terminal body weight in males at 20,000 ppm, significantly increased mean absolute and relative liver and spleen weights in males and 2000 ppm and in males and females at 20,000 ppm. **Gross necropsy**: increased incidence in enlarged spleen males and females at 2000 or 20,000 ppm, increased incidence or severity of pigment accumulation in liver, spleen and kidney as well as increased extramedullary hematopoiesis in spleen of males and females at 2000 or 20,000 ppm.			
Rats, 6 males and 6 females per dose group (NOS)	0, 50, 250, 1000, 2500, or 10,000 ppm ai technical in diet for 2 weeks	No effects at 50 or 250 ppm target organ = hemopoietic system at 1000 ppm, observations included decreased RBC (females), hemoglobin (females), and hematocrit (both sexes); increased liver weight (females) and liver-to-body weight ratio (both sexes). at 2500 ppm, additional effects included increased spleen weight (females) and spleen-to-body weight ratio (females) at 10,000 ppm (~700 mg/kg/day), additional effects included decreased food consumption, body weight (males), RBC (males), and hemoglobin (males); increased spleen weight (males) and spleen-to-body weight ratio (males). Effects at higher doses generally more severe than those observed at lower doses; no effects on survival or body weight (females), and no clinical signs of toxicity or gross pathology	Hazleton and Quinn 1995b MRID 43781708 (Hazard evaluation)

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference
Rats, 10 males and 10 females (NOS)	0 or 20,000 ppm ai in diet for 4 weeks; (20,000 ppm approximates limit dose of 1000 mg/kg/day)	Decreases observed in body weight, body weight gain, food consumption, RBC, hemoglobin, and hematocrit. Males showed increased liver and spleen weights (absolute and relative to body weight). There were no effects on survival and no clinical or gross signs of toxicity. This study together with the 2-week range finding test was used to select doses for the 13-week study.	Hazleton and Quinn 1995b MRID 43781708 (Hazard Evaluation and toxicity summary)

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference
Rats, CD, ~4-weeks old, 10 males and 10 females per dose group	0, 20, 200, 2000, or 20,000 ppm in diet for 13 weeks	No mortality; no adverse neurobehavioral, clinical, ophthalmoscopic, or gross necropsy findings. **Body weight**: statistically significant decrease at weeks 4 and 13 in females at 2000 ppm and in males and females at 20,000 ppm; body weight gain values significantly decreased at weeks 0-4 and 0-13 in males and females at 2000 or 20,000 ppm; food consumption significantly decreased at weeks 1-4 in males and females at 2000 or 20,000 ppm. **Hematology**: significant decreases in mean erythrocyte count, hemoglobin , and mean cell hemoglobin values as well as significant increases in mean cell volumes in males and females at 2000 or 20,000 ppm; decreased hematocrit and platelet values and increased mean cell hemoglobin and reticulocyte values in 20,000 ppm females; decreased myeloid/erythroid ratio in 2000 ppm females (with slight but not significant decrease in males and females at 20,000 ppm); significant increases in mean glucose and globulin values in females at 20,000 ppm. **Organ weights**: significantly decreased terminal body weight value for females at 2000 ppm and for males and females at 20,000 ppm; increased absolute liver weight in females at 20,000 ppm; increased spleen-to-body weight values in males and females at 20,000 ppm; increased liver-to-body weight values in females at 2000 ppm and males and females at 20,000 ppm; increased liver-to-brain weight value in females at 2000 or 20,000 ppm. **Histomorphology**: increased severity of splenic pigmentation in males and females at 2000 or 20,000 ppm. **NOEL (dietary administration for 13 weeks) = 200 ppm**	Osheroff 1991b MRID 42436219 MRID 43781708 (data summary)

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference
CHRONIC			
Dogs, beagles, purebred, 6- to 7-months old, weighing: 7.00-10.55 kg (males) and 5.75-9.05 kg (females), 4 males and 4 females per dose group	oral administration by admixture of 0, 15, 50, 250, or 1500 ppm for 52 weeks. Based on measured food consumption, these dietary concentrations corresponded to doses of 0.4 to 0.7 mg/kg bw (15 ppm), 1.5 to 2.4 mg/kg bw (50 ppm), 6.4 to 11.3 mg/kg bw (250 ppm), and 42.8 to 71.1 mg/kg bw (1500 ppm)	No clinical signs of toxicity associated with treatment; no adverse effects at ≤50 ppm; slight reduction in body weight gain (in the absence of any effect on food consumption) in males at 1500 ppm. At 250 and 1500 ppm, a direct effect of treatment on red blood cells was indicated by the presence of Heinz bodies and an increase in levels of methemoglobin, which resulted in the increased destruction of red blood cells in the liver (histologically associated with an increase in Kupffer cell pigment) and spleen. The increased destruction of red blood cells most likely accounted for the statistically significant increase in liver/body weight ratio in males at 1500 ppm and the increased spleen weights in dogs exposed to 250 and 1500 ppm. Also consistent with the effect of increased red blood cell destruction is the increase in plasma bilirubin at 250 and 1500 ppm.	Richards 1992a,b MRID 42931203 MRID 42931204

Additional Notes on Richards 1992a,b:
Other adverse effects included decreases in red blood cell counts, hemoglobin concentrations, and packed cell volume, compensatory increased in red blood cell production, minimal hemopoiesis in the spleen and hyperplasia in the sternal and femoral bone marrow, and increases in platelet and reticulocyte counts. All of these effects, which were observed consistently at 1500 ppm and to a lesser extent at 250 ppm, are indicative of low grade hemolytic anemia.

The increase in methemoglobin levels evidenced a statistically significant dose-response relationship at weeks 13, 15, 21, 39, and 52. [Table 5.1, p. 86. Fiche of this table is very difficult to read. Durations are taken from section 3.7, p. 23.] Based on comparisons to the control group, however, only the high dose group male dogs had a statistically significant increase by the end of the study, 1.7% in exposed group compared to 0.9% in the control group.

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference
Mice, Crl:CD-1, ~6-weeks old, weighing 23-33 g (males) and 17-26 g (females), 60 males and 60 females per dose group	nominal dietary concentrations of 0, 5, 50, 500, or 1000 ppm ai for 18 months, corresponding to overall compound consumption of 1, 8, 78, or 155 mg/kg/day (males) or 1, 9, 94, or 186 mg/kg/day (females).	NOEL = 50 ppm [8 mg/kg/day (males) and 9 mg/kg/day (females). No oncogenic effects at dietary levels up to 1000 ppm (equivalent to intake of 155 and 186 mg/kg/day for males and females, respectively); no adverse effects on body weight, body weight gain, food consumption, or food efficiency; treatment related effects indicative of chronic toxicity included hematological changes and spleen histopathology at 500 or 1000 ppm. Decreased survival in males at 500 and 1000 ppm and in females at 1000 ppm was judged to be an equivocal finding based on historical control data and lack of associated pathologies.	Trutter 1992a MRID 42931205 Trutter 1992b MRID 42931206
Rats, CRL:CD, ~6-weeks old, 70 males and 70 females per dose group	0, 10, 100, 1000, or 2000 ppm in diet for 24 months (interim sacrifice at 12 months); overall compound consumption values for males: 0.5, 5, 48, or 97 mg/kg/day, and for females: 0.6, 6, 61, or 125 mg/kg/day	no treatment related effect on survival; no oncogenic effects; treatment-related effects indicative of chronic toxicity at 1000 or 2000 ppm included decreased mean body weight and body weight gains, hematological effects (e.g., decreases in mean erythrocyte count, hematocrit and hemoglobin counts), and spleen histopathology (e.g., statistically significant increase in spleen-to-body weight ratio in high dose females, likely related to hematology findings). NOEL = 100 ppm (5 and 6 mg/kg/day for males and females, respectively)	Trutter 1992c MRID 42931208

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference
REPRODUCTION/TERATOLOGY			
Rabbits, New Zealand white, pregnant females, 5.5- to 6-months old, 20 per dose group	0, 50, 250, 1000 mg/kg/day once daily by gavage on day 7-19 of gestation; vehicle: aqueous 0.5% (w/w) sodium carboxymethyl-cellulose	No treatment-related deaths or clinical signs of toxicity; no treatment-related effects on maternal body weight or food consumption; no signs of maternal or developmental toxicity at any dose level. NOEL = 1000 mg/kg/day (highest dose tested)	Swenson and Solomon 1992 MRID 42436227
Rats, Sprague-Dawley, pregnant females, 25 per dose group.	0, 50, 250, or 1000 mg/kg/day once daily by gavage on days 6-15 of gestation; vehicle: aqueous 0.5% (w/w) sodium carboxymethyl-cellulose	No mortality; no clinical toxicity or adverse findings at necropsy. **At 1000 mg/kg/day**: reduced maternal body weight gain on days 6-20 of gestation (after correction for gravid weight); decrease in relative food consumption on days 7-8 and 6-9 of gestation, significantly reduced ($p \leq 0.05$) on days 8-9 of gestation. No effects on litter averages for corpora lutea, implantations, sitter sizes, live fetuses, early and late resorptions, or the number of dams with any resorptions. No developmental effects occurred at the high (1000 mg/kg/day) dose. NOAEL = 250 mg/kg/day.	Hoberman 1991 MRID 42436225
Rats, Crj:CD, ~5-weeks old, 24 males and 24 females per dose group	0, 25, 200, or 2000 ppm in diet for two consecutive generations	no reproductive effects at concentrations ≤2000 ppm systemic toxicity observed in parental rats (i.e., adverse effects on hemopoietic system and body weight effects) at concentrations ≥200 ppm NOEL (for reproductive effects) = 2000 ppm ai (149-195 mg/kg/day in males and females, respectively) NOEL (for systemic toxicity) = 25 ppm ai (1.9-2.3 mg/kg/day for males and females, respectively)	Aso 1995 MRID 43797701 Hazleton and Quinn 1995b MRID 43781708 (Hazard evaluation and data summary)

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference
Rats, CRL:CD, ~6-weeks old, 25 males and 25 females per dose group	0, 10, 150, or 2000 ppm in diet through two generations	NOEL (for reproductive effects) = 150 ppm (11.5-13.6 mg/kg/day for males and 12.8-14.5 mg/kg/day for females)	Danberry et al. 1993 MRID 42931207 Hazleton and Quinn 1995a MRID 43781707

Additional Details from Danberry et al. 1993: No treatment related mortality or clinical signs of toxicity in any generation at any dose level; ≤150 ppm did not cause effects on body weights or food consumption in any generation; 2000 ppm caused a decrease in body weight and food consumption in P_1 and P_2 males; histopathological changes in the spleen and toxicity of the hemopoietic system in rats of both sexes from both generations were consistent with the general pattern of toxicity observed in other non-developmental/non-reproductive studies

There were no treatment-related effects on mating or fertility in either generation at any dose level; there were no treatment related effects on reproduction in either generation at 10 or 150 ppm; **at 2000 ppm, there was an increased incidence of mortality of females during delivery (P_2), an increase in gestation length (P_2), a decrease in the mean number of implantation sites per female (P_2), and an increased incidence (equivocal) of pregnant females that did not deliver (P_1 and P_2).**

There were no treatment related effects on any offspring with respect to body weights, viability, malformations, or variations.

Hazleton and Quinn 1995a (MRID 43781707) conclude that dietary concentrations ≤2000 ppm tebufenozide do not cause reproductive effects in rats; NOEL = 149-195 mg/kg/day in males and females, respectively; NOEL for toxicity = 25 ppm (1.9-2.3 mg/kg/day in males and females, respectively

Appendix 3: Dermal, inhalation, and ocular effects of tebufenozide in experimental mammals

Animal	Dose/Exposure	Response	Reference
DERMAL			
Rats, CD, adults, 6 males and 6 females	2.0 g/kg bw undiluted Mimic®240 LV applied to shaved intact skin and occluded for 24 hours, after which the application sites were wiped with paper towels saturated with tap water and blotted dry.	No mortalities, clinical signs of toxicity, or body weight effects. Red stains observed on the fur surrounding the eyes and muzzle of several animals were attributed to test methods and use of collars. Skin irritation, manifested as erythema, edema, dessication, and scabs, was observed; however, necropsy revealed no gross changes. Acute dermal LD_{50} >2.0 g/kg bw Rohm and Haas classifies the test formulation as "PRACTICALLY NON-TOXIC by single dermal exposure" This study reveals the components of in the formulation. This information cannot be released	Parno and Gingrich 1994a MRID 44727703
Rats (NOS)	5.0 g/kg technical, single dermal application	"practically non-toxic;" no treatment-related mortalities or signs of toxicity at limit dose of 5.0 g/kg LD_{50}>5.0 g/kg	Hazleton and Quinn 1995b MRID 43781708 (Hazard evaluation/Toxi-city summary)
Rats, CD, adults, 6 males and 6 females	5000 mg/kg bw undiluted Mimic®240 LV applied to shaved intact skin and occluded for 24 hours, after which the application sites were wiped with paper towels saturated with tap water and blotted dry.	No mortalities, clinical signs of toxicity, or body weight effects. Dessication at the application site affected several of the animals beginning on day 3 and continuing until day 9; necropsy revealed no gross changes. Acute dermal LD_{50} >5000 mg/kg bw Rohm and Haas classifies the test formulation as "PRACTICALLY NON-TOXIC by single dermal exposure" This study reveals the components of in the formulation. This information cannot be released	Parno 1997 MRID 44727704
Rats, 10 males and 10 females per dose group (NOS)	0 or 1000 mg ai/kg bw/day semi-occlusive 6-hour dermal exposure, 5 days/week for 4 weeks or 0, 62.5, 250, or 1000 mg ai/kg bw/day.	NOEL (dermal application for 4 weeks) = 1000 mg ai/kg bw/day No treatment-related effects on hematology or clinical chemistry parameters, organ weights, gross pathology or histopathology at any dose level	Hazleton and Quinn 1995b MRID 43781708 (Hazard Evaluation/data summary)

Appendix 3: Dermal, inhalation, and ocular effects of tebufenozide in experimental mammals

Animal	Dose/Exposure	Response	Reference
Rats, Crl:CD, adults, 6 males and 6 females per dose group	Daily dermal applications of RH-75,992 2F formulation and RH75,992 technical or skin of rats for 4 weeks at doses up to and including 1000 mg ai/kg/day.	NOEL = 1000 mg ai/kg No treatment-related systemic effects; minor dermal irritation observed in females were attributed to RH-75,992 2F formulation solvent and not the active ingredient.	Morrison et al. 1993 MRID 42991507
Rabbits, New Zealand white, adults, 6 males	0.5 mL undiluted Mimic®240 LV applied to shaved intact skin and sites were semi-occluded for 4 hours, after which the application sites were wiped with paper towels saturated with tap water and blotted dry.	No mortalities or clinical signs of toxicity. At 1 hour, well-defined erythema was observed in all rabbits (6/6). Observed erythema ranged from well-defined to none among rabbits at 24, 48, and 72 hours but was no longer evident by day 7. Edema was not observed during the study. Rohm and Haas classifies the test formulation as slightly irritating to skin. This study reveals the components of in the formulation. This information cannot be released	Parno 1997 MRID 44727704
Guinea pigs, Hartley, young females, 20 treated, 10 positive controls, 10 naive controls	Skin sensitization protocol as detailed in the first row of the next page.	No significant erythema observed in any of the guinea pigs induced with mimic formulation; 100% incidence of erythema in positive control group; no erythema in naive control group. Mimic did not produce delayed contact hypersensitivity in guinea pigs in this study. This study reveals the components of in the formulation. This information cannot be released	Anderson and Shuey 1994

Anderson and Shuey 1994 ***Exposure details:***
Induction: treated guinea pigs received three 6-hour induction doses (1 dose/week for 3 consecutive weeks) of 0.4 mL undiluted Mimic®240 LV to shaved skin; positive controls received three 6-hour induction doses (1 dose/week for 3 consecutive weeks) of 0.4 mL DNCB (1600 ppm in 80% aqueous ethanol). **Challenge dose**: 2 weeks after the last induction dose, treated pigs received 0.4 mL undiluted Mimic®240 LV and positive controls received 0.4 mL DNCB (800 ppm in acetone). Naive control group received 0.4 mL undiluted Mimic®240 LV to shaved skin at one site and 0.4 mL DNCB (800 ppm in acetone) at a separate site.

Appendix 3: Dermal, inhalation, and ocular effects of tebufenozide in experimental mammals

Animal	Dose/Exposure	Response	Reference
Guinea pigs, young adults, albino, 20 (test group), 10 (control and positive control groups), 5 (positive control-naive control).	Test material administered as 5% w/w mixtures for intradermal injection and as 25% w/w mixture in petrolatum for topical induction and challenge applications	No skin sensitization in guinea pigs treated with test material; sulfathizole (used for positive control group) was shown to be an extreme sensitizer.	Glaza 1993 MRID 42991506
INHALATION			
Rats, 5 males and 5 females (NOS)	4.3 mg/L aerosol dust for 4 hours (NOS)	LC_{50}>4.3 mg/L (males) [0/5 deaths] LC_{50}>4.5 mg/L (females) [0/5 deaths] These were highest technically achievable concentrations.	Hazleton and Quinn 1995b MRID 43781708 (hazard evaluation)
Rats, Crl:CD, 6 males and 6 females	MIMIC wettable powder formulation. Mean aerosol concentration of 1.83 mg/L, nose-only exposure for 4 hours, followed by 14-day observation period	No mortality; no treatment-related clinical signs of toxicity or body weight effects; no treatment-related gross lesions observed at necropsy. LC_{50} >1.83 mg/L This study reveals the components of in the formulation. This information cannot be released	Bemacki and Ferguson 1994a MRID 44200306
Rats, CD, adults, 6 males and 6 females	4-hour nose only exposure to measured concentration of 1.33 mg/L Mimic®240 LV (nominal concentration = 178.2 mg/L The difference between the measured and nominal concentrations is attributed to the impaction of a portion of the aerosol on the interior surfaces of the exposure system.	No mortalities or body weight effects. Clinical signs included wet fur immediately after exposure, respiratory noise (1/6 males and 1/6 females), red-stained fur around eyes (1/6 males and 1/6 females), red-stained muzzle (1/6 males), tan-stained muzzle (5/6 males and 5/6 females). The tan stains (appearing to be test material) were attributed to poor positioning of the animals in the nose-only tubes. Tan stains, which appeared up to and including day 1 were not evident by day 2. Necropsy revealed the following changes: red pinpoint foci in the lungs (5/6 males, 1/6 females), slight to severe redness on all lobes of the lung (4/6 males and 6/6 females), which were considered to be consistent with irritation of the respiratory tract and judged to be treatment related. Combined male and female LC_{50} >1.33 mg/L	Bemacki and Ferguson 1994b MRID 44727705 This study reveals the components of in the formulation. This information cannot be released
OCULAR			

Appendix 3: Dermal, inhalation, and ocular effects of tebufenozide in experimental mammals

Animal	Dose/Exposure	Response	Reference
Rabbits (NOS)	direct application to corneal surface of eye or into conjunctival sac (NOS)	no irritation in eyes washed 30 or 60 seconds after dose or in treated eyes that remained unwashed RH-5992 technical calssified as "inconsequentially irritating to the eye."	Hazleton and Quinn 1995b MRID 43781708 (hazard evaluation and toxicity summary)
Rabbits, New Zealand white, adults, 6 males	0.1 mL undiluted Mimic®240 LV applied to conjunctival sac of one eye; untreated eye served as control. After 24 hour observation period, eyes irrigated with saline for approximately 60 seconds. Approximately 75% of test substance remained in contact with the eyes.	No mortality or clinical signs of toxicity. At 1, 24, 48, and 72 hours, positive corneal and conjunctival effects were observed in 2/6 rabbits; effects no longer evident by day 7. Rohm and Haas classifies Mimic®240 LV "MODERATELY IRRITATING" (i.e., a positive test that is reversible at ≥ 24 hours but ≤7 days.	Gingrich and Parno 1994s MRID 444727706

Appendix 4: Toxicity of tebufenozide to birds after oral administration.

Animal	Dose	Response	Reference
ACUTE			
Bobwhite quail, 13-days old, 10 per dose group	0, 312, 625, 2500, or 5000 ppm a.i. in diet for 5 consecutive days followed by a 3-day recovery period. Food consumption was about 13% of body weight during the exposure period (Tables III and IV). Thus, the dietary concentrations correspond to doses of 0, 41, 81, 325, 650 mg/kg bw/day.	LD_{50} >5000 ppm a.i.	Fletcher. 1990a MRID 42436235
Ducks, Mallard, 8-days old, 10 per dose group	0, 312, 625, 1250, 2500 or 5000 ppm in diet for 5 consecutive days followed by a 3-day recovery period	LD_{50} >5000 ppm a.i.	Fletcher 1990b MRID 42436237
LONGER-TERM			
Bobwhite quail, 29-weeks old, five males and five females per dose group	0, 1470, or 2150 mg a.i./kg via gelatin capsules for 21 days.	No mortality, no signs of toxicity, and no statistically significant difference in body weights, compared with controls. No abnormal tissue alterations were observed at necropsy. Acute LD_{50} >2150 mg a.i./kg bw	Fletcher 1987 MRID 42436234
Ducks, Mallard, 25-weeks old, 16 males and 16 females per dose group	0, 100, 300, or 1000 ppm ai in the diet for 20 weeks	No mortalities or treatment related adverse effects at any dose level; no adverse effects observed on body weight, food consumption, or reproductive endpoints. NOEL = 1000 ppm ai	Beavers et al. 1993a MRID 42991503

Appendix 4: Toxicity of tebufenozide to birds after oral administration.

Animal	Dose	Response	Reference
Bobwhite quail, 18-weeks old, 16 males and 16 females per dose group	0, 100, 300 or 1000 ppm ai in the diet for 20 weeks. Based on reported food consumption rates of about 15% of body weight (see special note below), the dietary concentrations correspond to doses of 0, 15, 45, and 150 mg/kg/day. See special note below.	No treatment-related mortalities, overt signs of toxicity, or effects on body weight or food consumption at any concentration. **Reproductive effects**: at 300 ppm, possible slight reduction in number of eggs laid (reflected in 14-day old survivors as % maximum eggs set and number of 14-day old survivors per hen per day A substantial drop in feed consumption was observed during weeks 8 and 9. At 1000 ppm, slight decreases in number of eggs laid and number of viable embryos. NOEL (for reproductive parameters) = 100 ppm	Beavers et al. 1993b MRID 42991501 Reinert et al. 1993a MRID 42991502

SPECIAL SUPPLEMENTAL NOTES ON BEAVERS ET AL. 1993b [MRID 42991501, MRID 42991502]
mg/kg bw doses: Average doses in units of mg/kg bw are not provided in the study. Table 2, p. 34. Average food consumption is estimated at 30 g per bird. There was a slight transient decrease food consumption at weeks 10 and 11 in all dosed animals and weeks 13/14 in the two higher dose groups. The magnitude of the decrease was about 16% to 33%below that of controls. The average body weights of the animals was about 200 g over the course of the study. Thus, food consumption is taken as 15% of body weight (30 g/200 g). The methods specifically state that food and water were available *ad libitum*. *"No attempt was made to quantify the amount of feed wasted by the birds, as the wasted feed is normally scattered and mixed with water and excreta."* (p. 16).

Effects: See Supplemental Table 1 at the end of this appendix.

Reinert et al. 1993a [MRID 42991502], which is a supplemental report indicates that two orders of magnitude difference between the NOEL for bobwhite quail (100 ppm) and mallard duck (1000 ppm) is not consistent and concludes that many of the endpoints in the bobwhite study are confounded by the usual variability in long-term studies and that the lack of dose-response in many parameters when judged against available data in avian studies does not support a conclusion of adverse effects at 300 ppm ai in the diet and that the NOEL probably approaches 1000 ppm, as supported in the mallard study.

Appendix 4: Toxicity of tebufenozide to birds after oral administration.

Animal	Dose	Response	Reference
Bobwhite quail, 18-weeks old, 15 males and 15 females per dose group	0, 150, 240, 385, or 615 ppm ai in diet for 20 weeks. Based on reported food consumption rates of about 8% of body weight (see special note below), the dietary concentrations correspond to doses of 0, 12, 19.2, 30.8, 49.2 mg/kg/day.	No treatment-related mortalities, overt signs of toxicity or effects on body weight or feed consumption; no apparent effects on reproductive endpoints. NOEL = 615 ppm (highest dose tested) LOAEC >615 ppm	Reinert 1995a MRID 43781701 Reinert 1995b MRID 43781702 (Supple-mental report) Reinert 1995c MRID 43781703 Supple-mental report of statistical analysis)

SPECIAL SUPPLEMENTAL NOTES ON REINERT 1995a,b [MRID 43781701 AND MRID 43781702]:
mg/kg bw doses: Average doses in units of mg/kg bw are not provided in the study. Table 3b, p. 24. Average food consumption is estimated at 16 g per bird. This is only about one-half of the food consumption in the Beavers et al. 1993b study - i.e., about 30 g/bird - summarized in the previous entry. The average body weights of the animals was about 200 g over the course of the study, similar to the body weights in the Beavers et al. 1993b study. Thus, food consumption is taken as 8% of body weight (16 g/200 g). The food consumption estimates did explicitly consider measurements of food wastage - i.e., food scattered from the container and not consumed. Ad libitum feeding is assumed but not specified.

Effects: See Supplemental Table 2 at the end of this appendix.

Supplemental Tables for Appendix 4

Appendix 4, Supplemental Table 1:
Details of reproductive parameters in bobwhite quail (from Beavers et al. 1993b, Table 3, p. 36)

Parameter	PPM in Diet			
	0	100	300	1000
Eggs Laid	714	769	570	508
Eggs Cracked	12	15	9	14
Eggs Set	627	680	496	435
Viable Embryos	595	616	451	367
Live 3-Week Embryos	592	609	451	367
Hatchlings	569	564	429	348
14-Day Old Survivors	544	516	387	322
Eggs Laid/Hen	48	48	38	36
Eggs Laid/Hen/Day	0.68	0.69	0.54	0.52
14-Day Old Survivors/Hen	36	32	26	23

Appendix 4, Supplemental Table 2:
Details of reproductive parameters in bobwhite quail (from Reinert 1995a, pp. 24-29)

Parameter	PPM in Diet				
	0	150	240	385	615
Eggs Laid	640	632	514	671	516
Eggs Cracked	2	2	1	0	0
Eggs Set	576	587	476	623	483
Viable Embryos - Day 5 Candeling	492	550	409	589	449
Viable Embryos - Day 11 Candeling	488	545	398	578	446
Live 18-Day Embryos	476	540	392	573	441
Hatchlings	449	474	345	522	408
14-Day Old Survivors	418	429	323	491	375
Eggs Laid/Hen	42.7	42.1	36.7	44.7	34.5

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Insects			
Honey bee, adult	0, 59, 117, and 234 µg/bee; 96 hour observation period.	Mortality rates in exposed bees were about 3.4% to about 5% and were less than control mortality (5.88%) NOEC = 234 µg/bee	Atkins 1990 MRID 42436244
Mite, predatory *Stethorus punctum*	Tests on larvae, pupae, and adults by 24-hour dry film exposures, with concentrations ranging from 9-90 ppm. Tests on eggs placed on treated leaves (92 ppm) Note: unclear if concentrations are concentrations of solutions leaves were dipped in or concentration on leaf material.	Not toxic to eggs, but survival of larva was reduced compared to untreated controls. Larval mortality likely due to contact with residues on leaf (not delayed effect of exposure during egg stage) In contact assay, tebufenozide was not toxic to adults and did not effect pupal survival. Less toxic than diflubenzuron.	Biddinger and Hull 1995
Tufted apple bud moth larvae (*Platynota idaeusalis*) [target species]	Dietary exposure.	7-Day LC_{50} = 1.63 ppm 14-Day LC_{50} = 1.12 ppm Somewhat lower LC_{50} values in sensitive laboratory strain.	Biddinger et al. 1998
Tufted apple bud moth larvae (*Platynota idaeusalis*) [target species]	Dietary exposure. 0.03 or 0.05 ppm	No effect on larval or pupal development. Decreased fecundity in matings when both sexes were exposed.	Biddinger and Hull 1999
Cydia pomonella codling moth [target species]	Dietary exposure.	LC_{50} = 0.025 ppm Dose-related decrease in number of viable eggs from exposed females, especially at concentrations > than the LD_{50}. No effect if males only were exposed. Dose-dependent decreased in time to emergence of adult insect from pupal case. Effect more pronounced in females than males.	Brown 1996
Hyssopus pallidus, Hymenopteran parasitoid on codling moth eggs	Exposure via codling moth exposed to up to 40 ppm tebufenozide in diet [24x LC_{50}]	No adverse effects on egg or larval development of parasitoid at 40 ppm tebufenozide [24x LC_{50}]	Brown 1996

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Ascogaster sp Hymenopteran endoparasitoid on codling moth eggs	Codling moth exposed to 40 ppm tebufenozide [24x LC_{50}]	LC_{50} = 0.07971 ppm, 3x LC_{50} values for moth	Brown 1996
Honey bee (*Apis mellifera*)	24-hour and 72-hour exposure by direct contact, indirect contact (test substance on filter paper) and inhalation to 0.1% v/v (equivalent to 1.05 kg/ha in 1000 L/ha) tebufenozide formulation Hoe 105540 SC (a 24% a.i. water soluble formulation) 3-hour (250 µg a.i./bee) feeding and 24-hour feeding (dose range approximately 2.4 to 800 µg a.i./bee) Note: for all contact and inhalation exposures, it is unclear is concentrations are given in terms of formulation or a.i. Authors state that 0.1% v/v is equivalent to twice the application rate	Direct exposure **24-hr**: 2% mortality in treatment group and 0% in controls **72-hr**: 14% mortality in treatment group and 12% in controls Indirect exposure **24-hr**: 0% mortality in treatment and control. **72-hr**: 10% mortality in treatment group, 8% in controls. Inhalation exposure **24-hr**: 0% mortality in treatment and 2% mortality in control **72-hr**: 10% mortality in treatment and control. Oral exposure **3-hr**: 0% mortality in treatment and control. **LD_{50} > 250 µg/bee** **24-hr**: 0% mortality in highest dose group. 2% mortality in controls. No dose-dependent mortality was observed. **LD_{50} > 800 µg/bee**. No behavioral effects noted for any route of exposure or duration of exposure.	Chan 1995 MRID 43797702
Honey bee (*Apis mellifera*)	tebufenozide formulation Hoe 105540 SC (a 24% a.i. water soluble formulation) applied at rate of 1.05 kg/300 L applied at rate of 0.2 kg/ha. [Appears to be given in terms of formulation, although this was not specifically stated]	Bee colonies tested in laboratory. No increased in treatment-related mortality was observed. No effects of treatment on flight activities or behavior. No effects on brood (as measured by dead pupae).	Chan 1995 MRID 43797702

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Trichogramma pretiosum (parasitic wasp)	Exposure to *T. pretiosum* by dipping parasitized host eggs of *Ephestia kuehniella* in solutions of tebufenozide. Eggs dipped for 5 seconds on tebufenozide solution of 25 g a.i./100 L.	Three different development stages of parasitized host eggs tested – egg-larvae, pre-pupae, and pupae. No significant increase in *T. pretiosum* mortality compared to untreated controls. Decreased development time was slightly significantly decreased for tebufenozide applied at the pupae stage (tebufenozide 9.68 days in control group and 9.35 day in tebufenozide group), but not when applied at the egg-larvae and pre-pupae stages. For parasite, parasitism capacity reduced when tebufenozide was applied at the egg-larvae and pre-pupae stages, but not when applied at the pupal stage,	Consoli et al. 1998
Mexican rice borer (*Eoreuma loftini*)	laboratory study. Exposure via leaves collected from sprayed field as follows: <u>1996 season</u> leaves collected 1 day after field application of low dose Confirm (0.14 kg a.i./ha) and high dose Confirm (0.2 kg a.i./ha). Insects were 1st instar larvae <u>1997 season</u> leaves collected 1 and 4 days after application of Confirm (rate of 0.28 kg a.i./ha). Insects were 2nd and 3rd instar larvae	<u>For the 1996 season</u> Cumulative mortality as follows: low dose: 34.4% high dose: 39.4% untreated control: 0% <u>For the 1997 season</u> For organisms exposed to leaves collected 1 day after field application: after 9 days of exposure, mortality was approximately 80% (data presented graphically). 100% mortality after 12 days of exposure For organisms exposed to leaves collected 4 days after field application: after 9 days of exposure, mortality was approximately 20% (data presented graphically). Mortality not assessed after 9 days.	Legaspi et al. 1999

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
braconid parasitoid *Allorhogas pyralophagus*	exposure via leaves collected 1 and 4 days after field after applications of Confirm in 1996 and 1997. 1996: low dose 0.14 kg a.i./ha and high dose0.2 kg a.i./ha 1997: 0.28 kg a.i./ha	Using 1997 field treatments [according to figure 5 legend, p 809], no mortality was observed in for *A. pyralophagus* exposed to leaves (collected 1 day and 4 days after field application) for 4 and 24 hrs. Using 1997 field treatments [according to figure 6 legend, p 809], no difference was observed between control and high dose tebufenozide, but longevity was decreased for low dose tebufenozide.	Legaspi et al. 1999

Note on Legaspi et al. 1999: From the methods section, it appears that 2 application rates of Confirm were tested in 1996 and one was tested in 1997. However, results for 1997 are presented for low and high dose groups.

Beet army worm, 3rd instar (Lepidoptera: noctuidae)	tebufenozide (Confirm 2F) in food at 22.7 % a.i. (wt/wt) after exposure to diet for 120 hours	Susceptibility of field collected insects (9 strains) compared to ECOGEN laboratory strain using LC_{50} values ECOGEN LC_{50}: 17.6 ppm Field organisms LC_{50} values range from 39.7 to 176.3 ppm	Mascarenhas et al. 1998

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
predatory lacewing adults (*Chysoperla carnea*)	tebufenozide (TEB), 18, 90 and 180 ng/insect, applied topically [authors note that 90 mg/insect is the maximum field recommended (MFRD) dose]	Tebufenozide did not fecundity and egg fertility. In contrast, diflubenzuron reduced egg hatchability to 0% (compared to control 87%). To explore differences, compared cuticle penetration, distribution and excretion of compounds.	Medina et al. 2002
	Diflubenzuron (DBB) applied at 150 (2xMFRD)	<u>Cuticle penetration</u>: DFB 16% TEB 26% <u>Excretion</u>: DFB 24.8% of penetrated amount excreted in feces in 7 days TEB aprpox, 50% of penetrated amount excreted in feces in 7 days For DFB, only very small amounts of dose recovered in ovaries and deposited eggs. No TEB detected in ovaries or deposited eggs.	
predatory lacewing 3rd instar lavae (*Chysoperla carnea*)	Topical application of tebufenozide (TEB, Mimic 24% a.i.) applied at 0, 90 and 180 ng a.i./insect and diflubenzuron (DFB, 25% a.i.) applied at doses ranging from 0.5-75 ng a.i./insect Authors note that for TEB, 90 ng/insect is the maximum field recommended dose (MFRD)	TEB had no effect on pupation, adult emergence, fecundity or egg fertility. DFB LD_{50}: 2.26 ng a.i./insect. At the lowest dose tested (0.5 ng a.i./insect), no effect on fecundity or egg fertility compared to control Presented results of cuticle penetration and excretion studies as summarized above for Medina et al. 2002	Medina et al. 2003

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Indian meal moth (*Plodia interpunctella*)	dietary exposure of 1st instar lavae to tebufenozide (RH-5992) at concentrations of 0, 0.1, 1, 5, 10, and 25 ppm for up to 31 days	Larvae monitored for weight and mortality until metamorphosis. Weight gain: No effect on wt gain at concentrations up to 1.0 ppm. Exposure to 5 and 10 ppm results in decreased wt gain. Exposure to 25 ppm results in larval weight loss. Mortality: At concentrations of 0.1 and 1 ppm, no effect on mortality. Mortality increased compared to control at concentrations 5 and 10 ppm. 100% mortality at 25 ppm. In cell culture (PID2 imaginal disc line), exposure to 0.005 µM tebufenozide significantly increased glucosamine uptake (increase by 30% of control level).	Oberlander et al. 1998
spruce budworm (*Choristoneura fumiferana*)	not reported in Keller and Brown 1998a summary	RH-5992 is effective in inducing a incomplete molt when fed to worms prior to appearance of the endogenous ecdysteroid peak, but when administered after the peak. However, incomplete molts are observed for subsequent molts, presumably due to the persistence of tebufenozide in cells.	Palli et al. 1995, as summarized in Keller and Brown 1998a
predaceous insidium flower bug (*Orius inisidoisus*), parasitic wasp *(Cotesia plutella*)	Confirm applied cotton plants at an application rate of 0.125 lb a.i./acre. Insects were tested on plants 2 and 24 hours after application. Insects exposed to fresh foliar residues for 24 and 48 hours.	O. insidoisus: exposure to 2- and 24-hour leaves for 24 or 48 hours did not results in an increase in mortality compared to control insects. C. plutella: no significant increase in percent mortality compared to control exposed to 2-hour old leaves.	Pietrantionio and Benedict 1999

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
spruce budworm (*Chorisroneura fumiferana*)	1-100 ng/insect tebufenozide by ingestion	In 6th instar insects, treatment induced lethal precocious molt. Lack of development of new cuticle due to lack of gene expression of dopadecarboxylase. Effect observed in 100% of insects administered a dose of 70 ng. For 4th and 5th instars, 100% effect for lethal precocious molt was observed at lower dose (20 ng/insect) Topical exposure did not induce effects at doses up to 10,000 ng/insect.	Retnakaran et al. 1997a
spruce budworm (*Chorisroneura fumiferana*), 6th instar stage	Insects force-fed 0.1 µg a.i. tebufenozide (aqueous flowable RH-5992)	Effects observed at time points after exposure: 6 hr – insects stop feeding. 12 hr – head capsule slips partially. 24 hr – pronounced head capsule slippage and mid-dorsal split of old cuticle. Insect remains in this state and ultimately dies of starvation and dessication. Microscopy if integument showed hypertrophy of golgi complex and alterations in the cutlicular components, and organelles of epidermal cells.	Retnakaran et al. 1997b
two lacewing species – *Chrysoperla carnea* (Stephens) and *Micromus tasaniae* (Walker)	Petri dishes sprayed with tebufenozide (Minic 20 flowable liquid) at concentations of 0.08 to 0.8 % a.i.) and film left to dry. To test for acetylcholinesterase activity (AchE), insects were exposed for 2 and 24 hours. For Glutathione-S-transferase (GST), insects were exposed for 10 hours.	For both species, no inhibition of head AchE or whole body GST.	Rumph et al. 1997a

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
lacewing *Micromus tasaniae* (Walker) (3rd instars)	Test materials applied to petri dishes. Tebufenozide 7.44 µg/cm^2(according to authors, this is 10x the recommended field rate). For tebufenozide-exposed larvae, effects in offspring were also examined, but offspring were not exposed to any test substance. Diflubenzuron (DFB) 0.07 µg/cm^2	Examined effects of tebufenozide and DFB on life-table parameters (sex ratio, longevity, sterility and fecundity) in adults derived from treated larvae. Tebufenozide: No mortality observed. No treatment effect for sex ratio, longevity or number of sterile pairs for either first or second generation. Total number of eggs in reduced by 30% in 2nd generation, but not 1st generation. Decreased in oviposition period for 1st generation (33.3 days) and 2nd generation (30.5 days), compared to control (39.8 days). Only 2nd generation change significant. No change in preoviposition period for either generation. DFB: Higher proportion of females in DFB (64.9%) compared to controls (53.0%). Longevity reduced for females in DFB (34.1 days) compared to controls (46.1 days). No treatment effect for in number of sterile pairs, although a strong trend observed toward an increase in infertility. Daily number of eggs reduced. Increased preoviposition period. Significant decrease in oviposition period.	Rumph et al. 1998
Codling moth (*Cydia pomonella*) – 3 strains	Tebufenozide (Confirm) dose range 10-10,000 ng/insect, applied topically	In susceptible strains of diapausing larvae, tebufenozide breaks the diapausing period and induces molting and reduces the pre-emergent period. In resistant strains, treatment did not break the diapausing state. LC_{50} values of various strains – Sv: 27.4 ng/insect Rv: 362 ng/insect Rt: 1570 ng/insect	Sauphanor et al. 1999

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Larvae of *Galleria*, *Sarcophaga* and *Calliphora*	topical application of RH-5992 (dose range not specified in Keller and Brown 1998a summary)	*Galleria*: stimulation premature molt. ED_{50} = 1.75 µg/insect *Sarcophaga* and *Calliphora*: did not induce molt	Slama 1995, as summarized in Keller and Brown 1998a
Spodoptera exempta (Walker) (beet armyworm), *Spodoptera exigua* (Hubner) (beet armyworm), *Spodoptera littoralis* (Egyptian armyworm), *Mamestra brassicae* (cabbage moth), *Galleria mellonella* (greater Wax moth)	Exposure by topical or oral routes. Topical application of 0.01 to 40,000 ng/insect. Oral exposure by feeding leaves or prey dipped in tebufenozide solutions or tebufenozide in honey water (technical grade tebufenozide)	*S. exempta* **LD_{50} (topical application):** 6.75 mg/insect for 6th instar **LC_{50} (fed dipped leaves** - values are concentration of test material leaves were dipped in) 3rd instar 0.034 mg/L 4th instar 0.095 mg/L 5th instar 0.085 mg/L 6th instar 0.084 mg/L *S. exigua* **LD_{50} (topical application):** 59.2 mg/insect for 5th instar **LC_{50} (fed dipped leaves)** 1st instar 9.7 mg/L 2nd instar 10.5mg/L 3rd instar 8.5mg/L 4th instar 10.0 mg/L 5th instar 2.5 mg/L Dose-dependent decrease in fecundity following oral exposure to tebufenozide in honey water (1, 10, and 100 mg/L), although all deposited eggs were viable *S. Littoralis* **LD_{50} (topical application):** 11.02 mg/insect for 6th instar *M. brassicae* **LD_{50} (topical application):** 8.53 mg/insect for 6th instar *G. mellonella* **LD_{50} (topical application):** 571 mg/insect for 6th instar For Lepidoptera larvae, tebufenozide induced lethal molt within 24 hours of exposure. Other effects included inhibition of weigh gain and feeding, extrusion of hindgut, and loss of hemolymph.	Smaggje and Degheele 1994a

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
larvae of *Leptinotarsa decemlineata* (Colorado potato beetle), *Diabrotica virgifera virgifera* (western corn rootworm), *Locusta migratoria migratoria* (migratory locust), and nymphs of *Podisus sagitta* (predatory stink bug)	Exposure by topical or oral routes. Topical application of 0.01 to 40,000 ng/insect. Oral exposure by feeding leaves or prey dipped in tebufenozide solutions or tebufenozide in honey water (technical grade tebufenozide)	No activity observed in any species at any dose or concentration tested.	Smagghe and Degheele 1994b
Spodoptera exempta (Afrian army worm), *Spodoptera exigua* (beet armyworm), *Lepinotarda decemlineata* (Colorado potato beetle)	For LC_{50} determination, insects were fed leaves dipped in tebufenozide (technical grade) solutions.	**LC_{50} values** (last instars) *S. exempta*: 0.034 mg/L *S. exigua*: 2.5 mg/L *L. decemlineata*: no mortality at concentrations up to 50 mg/L. At 100 mg/L, sings of neurotoxicity (tremor and paralysis) were noted. For *S. exempta* and *S. exigua*, dose-dependent decreased in larval weights. No affect of treatment on larval weight for *L. decemlineata*. Resistance of *L. decemlineata* and differences in sensitivities of *S. exempta* and *S. exigua* apparently not due to differences in pharmacokinetics. All three species showed similar pharmacokinetic parameters for absorption, excretion, distribution and metabolism of tebufenozide	Smagghe and Degheele 1994b

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Podisus nigrispinus and *P. Maculiventris* (predatory soldier bugs)	nymphs exposed orally to RH-5992 via feeding on larvae of *Spodoptera exigua* treated with 20 µg/larvae or in drinking water (100 mg/L) or exposed topically to up to 100 µg/nymph. Adults treated orally via feeding on larvae of *Spodoptera exigua* treated with 20 µg/larvae or in drinking water (100 mg/L)	No effect in either species for any exposure. No chemosterilizing effects observed in adults	Smagghe and Degheele 1995, as summarized in Keller and Brown 1998a
Cotton leafworm (*Spodoptera littoralis*), laboratory strain and field strain	tebufenozide (RH-5992 2F flowable) For repeated exposures to induce tolerance, exposure was dietary via leaves dipped in 0.6 mg a.i./L tebufenozide solution. For LC_{50} determination, tebufenozide applied uniformly to food [unclear if concentrations are final concentration in food or concentration of fluid applied to food.]	Repeated exposure over 5 generations did not result in the development of tolerance to tebufenozide. For 3rd instar insects, laboratory strain (LC_{50} 2.47 mg/L) was more susceptible than the field strain (LC_{50} 11.31 mg/L).	Smagghe and Degheele 1997
Spodoptera exigua (beet armyworm) and *Leptinotarsa decemlineata* (Colorado potato beetle)	Dietary exposure via leaves dipped in solution of 3 mg a.i./L tebufenozide (technical grade) for *S. exigua* and 50 mg a..i/L tebufenozide	*S. exigua*: In control insects, major hemolymph ecdysteroid peaks appeared ~3-4 days. After treatment with tebufenozide, hemolymph ecdysteroid peaks was abolished. Treatment resulted in decreased weight gain. Typical precocious molting observed. *L. decemlineata*: In control insects, major hemolymph ecdysteroid peaks appeared ~8-9 days. Peak unaffected by tebufenozide treatment. No affect of treatment on larval weight gain. No precious molting observed.	Smagghe et al. 1995

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Chrysodeixis chalcites (tomato looper), last instar	exposure to diet containing 100 µg a.i./g diet tebufenozide RH-5992 2F	Symptoms of premature molting observed within 12 hours of treatment. Significant reduction in larval weight and feeding. Ultrastructural changes of the integument included increase in endoplasmic reticulum, hypertrophy of golgi complex, increase in nuclear volume, numerous oval and elongated mitochondria. Prothoracic gland cells were reduced in size, show loss of cell organelles, and autophagic vacuoles appeared. In foregut epithelium, prominent vacuoles formed and most cell oragnelles disappeared. Ultrastructural changes also observed in muscle cells, with absent mitochondria.	Smagghe et al. 1997
Spodoptera exigua (beet armyworm)	Exposure via artificial diet with concentrations of tebufenoxide varying according to generation. G_{0-5}: 0.5 mg/L G_{6-10}: 1 mg a.i./L $G1_{1-12}$: 2 mg a.i./L For disposition studies, all insects were exposed to the same amount of test material (20,000 dpm) consumed on leaf material.	Continuous exposure of all larval instars to LC_{25} doses for over 12 generations revealed no loss in susceptibility for up to 5 generations. From G_4 onwards, generation-dependent reduction in oviposition. For G_4, 65% of G_0 oviposition, for G12, 0% oviposition. Higher tissue concentrations of 14C-tebufenozide in hemolymph, carcass, and gut in susceptible larvae compared to G_0 larvae. All insects were exposed to the same amount of test material (20,000 dpm consumed on a leaf).	Smagghe et al. 1998
Spodoptera exigua (beet armyworm) and *Ostrinia nubilalis* (European corn borer)	*Spodoptera exigua* exposed to tebufenozide in diet. 50 µL of solution containing 1 mg/L tebufenozide (50 ng) added to artificial diet in culture dish for exposure to 1 insect. *Ostrinia nubilalis* exposed to tebufenozide (0, 10, 25, 50, 200, 300, and 400 ng/insect) by injection.	*Spodoptera exigua* (last instar): Chitin formation in cuticle was increased in tebufenozide treated insects compared to controls. Treated insects died by day 3 after exposure *Ostrinia nubilalis* (day-1 male pupae): Tebudenzide exposure prevented the completion of adult development and eclosion. Time to death decreased with increasing dose. Tebufenozide exposure induced premature chitin synthesis in male claspers.	Smagghe et al. 1999a

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Spodoptera exigua (beet armyworm), last instars	Tebufenozide applied topically to individual insects. Mortality counts made 7 days after exposure.	LD_{50} = 7.06 mmole/insect	Smagghe et al. 1999b
Cydia pomonella (codling moth)	Exposure of adults to surfaces treated with tebufenozide solution (360 ppm*) throughout their lives, including mating and ovipositing). Recently emerged moths exposed to treated surfaces (360 ppm*) for 24 hours, then mated with unexposed partner (oviposit on non-treated surface) tebufenozide was RH-5992, 2F (flowable) * authors state that this is the recommended field rate	Continuous exposure to tebufenozide-treated surfaces resulted in significant reduction in number of eggs laid (control, 74.5 eggs; treatment 39.6 eggs) and number of eggs hatched (control, 58.4%; treatment, 6.6%). 24-hour exposure of females mated to unexposed males resulted in reduction in fecundity (control, 97.7 eggs; treatment 26.8 eggs) and fertility (control, 86.3%; treatment, 78.7%). No effect if exposed male was mated with unexposed female	Sun and Barrett 1999
Orius laevegatus (predatory bug)	exposure to plates sprayed with tebufenozide at the manufacturers recommended rate	No effect on development of nymphs or on oviposition.	van de Veire et al. 1996, as summarized in Keller and Brown 1998a
Gypsy moth [target species]	Tebufenozide applied to branches of oak trees at rate of "237 mL per 189 L final solution (label recommends 8 oz per 50 gal solution per acre), with 0.25 5 (v/v) Bond sticker". Difubenzuron (DFB) "Dimilin 25W at 237 mL per 378 L final solution, without added sticker".	Laboratory-reared gypsy moth larvae (1^{st}, 2^{nd}, 3rs, and 4^{th} instars studied separately) were placed in bags and tied onto tips of treated branches 1 hour after spraying. Larvae were exposed for 7-21 days. Same protocol was followed for larvae applied to branches 1, 2, 7, 14, 21, 28 and 35 days after spraying. For the exposure 1-hour post-application, 100% mortality observed for all insects after 21 days of exposure. Similarly, 100% mortality observed for all "aged" residues. DFB also showed very high efficacy, except for 69% mortality on 14-day residue. However, all other DFB aged residues resulted in 100% mortality.	Webb et al. 1998

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Epiphyas postvittana (lightbrown apple moth)	larvae exposed to tebufenozide (Mimic 70W) in food at concentrations of 0, 0.5, 1. 1.5, 2, 2.5, 3, 10, 30, 100, and 200 ppm.	Dose-mortality response determined at each larval stage. 1st instar: no survival to pupation at concentrations ≥1.5 ppm 3rd instar: no survival to pupation at concentrations ≥2.5 ppm 5th instar: dose-related decrease in survival to pupation. In 200 ppm exposure group, 14.8% survival. Time to mortality was less than in1st and 3rd instars. Mortality increased with increasing exposure time. Time to mortality for 3rd and 5th instars decreased when insects were exposed at 40◦C compared to 20◦C. 3rd instars more susceptible at higher temperature than 5th instars.	Whiting et al. 1999
Soil Invertebrates			
Earthworm *(Dendrobaena octaedra)*, 40 per dose	Deciduous leaves at 0 (untreated), 10X and 100 X EEC for 12 weeks. 55.4 ppm and 554 ppm based on reported EEC of 5.5461 mg/kg (equivalent to the application rate of 70 g/ha).	No effects on growth or reproduction (numbers or proportion hatching)	Addison 1996
Collembola (*Folsomia cundida*, *F. nivalis*, *Onychiurus parvicornis*, and *Hypogastrura pannosa*)	1996Coniferous substrate at 72.1 µg/g (ppm) organic matter for 8 to 10 weeks	No effect on survival or reproduction.	Addison 1996
Round worm larvae *(Ascaris suum)*	RH-5992 at concentrations in media of 5 and 50 ng/mL	Treatment had a biphasic effect on larval growth after 24-hour, premolt exposure – low concentrations (5 ng/mL) increase growth. Higher concentrations decreased growth (≥ 50 ng/mL)	Fleming 1998, as summarized in Keller and Brown 1998a

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
earthworm (*Eisenia foetida*)	14-day exposure to RH-5992 at soil concentrations of 0, 61, 140, 270, 580, and 1000 mg a.i/kg (Although not specified, assume this is kg soil).No effect on survival at any concentration tested.	14-day LC50 > 1000 mg ai/kg 14-day NOAEC >1000 mg ai/kg	Garvey 1992, as cited in Keller 1994 (MRID 43367001)

Appendix 6. Terrestrial field/mesocosm studies on tebufenozide

Application	Species Examined	Effects	Reference
Mimic 2F, 0.03 lb a.i./acre in mixed oak forest, May 1994	Gypsy moth; Other macrolepidoptera richness and abundance	Examined effect of treatment on richness and abundance of arthropod family and macrolepidoptera. Sampling conducted May-Aug 1994 and May-Aug 1995. Marginal decrease in gypsy moth populations (not statistically significant compared to control plots). Nontarget arthropod richness and abundance: except for macrolepidoptera families, no effect of treatment for either sampling year. Significant decrease in the microlepidopteran Gelechiidae ($p=0.02$) in treatment year but not following year Marginal ($p=0.07$) decrease in sap-feeding Tingidae in treatment year but not following year. Macrolepidoptera richness: no effect of treatment in either sampling year (compared to control). Macrolepidoptera abundance: decreased during the last 8-13 weeks of 1994, but not different from control in the first 1-7 weeks of 1994 or for any sampling period in 1995.	Butler et al. 1997

Appendix 6. Terrestrial field/mesocosm studies on tebufenozide

Application	Species Examined	Effects	Reference
Mimic 2F, 0.06 lb a.i./acre in mixed oak forest, May 1994		Examined effect of treatment on richness and abundance of arthropod family and macrolepidoptera. Sampling conducted May-Aug 1994 and May-Aug 1995. Marginal decrease in gypsy moth populations (not statistically significant compared to control plots). Nontarget arthropod richness and abundance: except for macrolepidoptera families, no effect of treatment for either sampling year. Significant decrease in the microlepidopteran Gelechiidae ($p=0.02$) in treatment year but not following year Marginal ($p=0.07$) decrease in sap-feeding Tingidae in treatment year but not following year. Macrolepidoptera richness: decreased during the first 1-7 weeks after treatment in 1994 and during the first 1-8 weeks of the 1995 sampling period (compared to control). Macrolepidoptera abundance: decreased for the 1994 season and for the first 1-8 weeks of 1995 season.	Butler et al. 1997
Additional Notes on Butler et al. 1997: Some macrolepidoptera (e.g., *Melanolophia canadaria*) were relatively insensitive while others (*Lophocampa caryae* [Hickory Tussock moth]) were highly sensitive.			
Mimic 2F, 70 and 140 g/ha [0.06 and 0.12 lb a.i./acre]	Spruce budworm	Larval survival not significantly decreased at one application of 70 g/ha. Significant reductions at two applications at 70 g/ha or one application at 140 g/ha. Phenological development and larval and pupil weights significantly decreased in treated budworms compared to untreated controls.	Cadogan et al. 1997

Appendix 6. Terrestrial field/mesocosm studies on tebufenozide

Application	Species Examined	Effects	Reference
Mimic, tested on apple plots in Australia 1994/1995 season: 8 applications of 15 g a.i./100 L applied by air-blast sprayer at 1720 L/ha [258 g a.i./ha or 0.23 lb/acre] 1995/1996 season: 9 applications of 10.5 g a.i./L applied by air-blast sprayer at 1720 L/ha [180.6 g a.i./ha or 0.16 lb/acre]	lepidopteran pests and nontarget arthropods and	Note: no untreated control plot. All comparisons were made to plots treated with other insecticides (azinphos-methyl and fenoxycarb). All plots treated with Mimic showed effective control over lepidopteran pests (codling moth, lightbrown apple moth, and early seasons caterpillars) Populations of natural enemies (increased spiders, lacewings, and the specialist preditor mite *Stethorus* spp. adults and larvae.	Gurr et al. 1999
Mimic 240 LV. 0.07 a.i. kg/ha. Two aerial applications spaced 4 days apart in June 1994. Ontario Canada	Tennessee warbler nests, 6 in control plot and 5 in Mimic treated plot. Monitored number of eggs laid, percent hatch and growth of the hatchlings	Decreases in both the average number of eggs per nest (6.3 in the control area and 5.8 in the treated area) as well as the percent hatch (97.4% in the control area and 89.7% in the treated area). Based on the number of eggs, the differences in hatching were 37/38 in control plot and 26/29 in treated plot. Using the Fisher Exact test, the p-value is 0.21 – i.e., not statistically significant. Decrease in brooding time and increase in foraging times in Mimic treated plot were probably associated with decrease in prey.	Holmes 1998
Confirm 70W RH-5992 wettable powder applied to sugar cane plots in Texas. For the 1996 season, two application rates: 0.14 kg a.i./ha and 0.2 kg a.i./ha [0.12 lb/acre and 0.18 lb/acre]. For the 1997 season, 0.28 kg a.i./ha [0.25 lb/acre]	Mexican rice borer (*Eoreuma loftini*)	For all application rates for the 1996 and 1997 growing seasons - Treatment did not decrease the damage to cane caused by *E. Loftini* in either growing season. No increase in cane juice yield or quality in either growing season.	Legaspi et al. 1999

Appendix 6. Terrestrial field/mesocosm studies on tebufenozide

Application	Species Examined	Effects	Reference
Confirm 2F applied to plots of peanuts at rates of 0.125 and 0.24 lb a.i./acre. Treatment applied on Aug 7, 1998. Plots monitored on days 2, 5, 7, 10, 14 and 20 after application.	defoliating caterpillars and beneficial arthropods (not specified)	For defoliating caterpillars, the only decreased in numbers was observed for the high dose Confirm on day 3 (9% of control) after treatment. Only decrease in beneficial arthropods observed for low dose Confirm (315 of control) on Day 3 after treatment but not on subsequent days (5 to 15 DAT). For beet army worm, numbers were decreased for low (6% of control) and high (5% of control) application rates on day 3 after treatment.	Mulder and Prescott 1999a
Confirm 2F applied to plots of peanuts at 0.25 lb a.i./acre. Treatment applied on Aug 7, 1998.	potato leafhopper, defoliating caterpillars (corn earworm, beet armyworm, rednecked peanutworm, and beneficial arthropods (not specified)	Potato leafhopper numbers increased on day 14 after treatment (220% of control), but not days 7 and 20 Number of total defoliating caterpillars decreased on day 3 (52% of control) and day 7 (14% of control) after treatment. Number of beet aryworms decreased on day 7 (0% of control) after treatment. Number of beneficial arthropods not decreased at any time point.	Mulder and Prescott 1999b
Greenhouse study. Tebufenozide (RH-5992-2F) applied at 35, 70, 140 and 280 g a.i./ha to potted white spruce trees. [0.03, 0.06, 0.12, and 0.24 lb/acre]	spruce budworm (*Chorisroneura fumiferana*) exposed to trees for 10 days	Evaluated effectiveness of treatment by mortality and feeding rate of 4th instar insects (by counting number of droppings, i.e., frass pellets). After 10 days exposure, mortality was not increased compared to controls for any treatment group. However, feeding inhibition was apparent and similar for all treatment groups.	Retnakaran et al. 1997a

Appendix 6. Terrestrial field/mesocosm studies on tebufenozide

Application	Species Examined	Effects	Reference
Tebufenozide applied (RH-5992-2F) applied at 35, 70, 140 and 280 g a.i./ha [0.03, 0.06, 0.12, and 0.24 lb/acre] to 0.1 ha plots of white spruce trees in Zee Casault, Gaspe, Quebec.	spruce budworm (*Chorisroneura fumiferana*)	For plots treated with $\geq$70 g a.i./ha, population reduction was 100% For plots treated with 35 g a.i./ha, population reduction was 95%. For all tebufenozide treated plots, defoliation was 1-2%, compared to 13-16% in control plots.	Retnakaran et al. 1997a
Tebufenozide applied to apple plots in New South Wales, Australia.. Treatments applied between Nov to Feb over the 1992-1993 and 1993-1994 growing seasons. In each season, 8 applications of Mimic at rate of 15 g a.i./100 L (volume/acre or ha not indicated) using conventional air-blast sprayer. No untreated control plots.	Several species - codling moth, early fruit caterpillars (not specified), lightbrown apple moth, the predatory mites *Typhlodromus pyri* and *Typhlodromus occidentalis*, spiders (*Stetorus* spp) and apple dimpling bug nymphs (*Campylomma liebknechti*)	Comparisons of the effects of tebufenozide were made to 2 other treatments: azinphos-methyl and fenozycarb. No differences between treatments for fruit damage due to codling moth or early fruit caterpillars in either season. In the 1992-1993 seasons only, tebufenozide more effective than fenoxycarb on controlling damage due to lightbrown apple moth. Tebufenozide was ineffective in suppressing populations of the phyoseiids *Typhlodromus pyri* and *Typhlodromus occidentalis*. Compared to azinphos-methyl treatment, numbers of spiders (*Stetorus* spp) and apple dimpling bug nymphs (*Campylomma liebknechti*), numbers were higher in the tebufenzide-treated plots.	Valentine et al. 1996

Appendix 6. Terrestrial field/mesocosm studies on tebufenozide

Application	Species Examined	Effects	Reference
balsam firm tree plots in Newfoundland One application tebufenozide (Mimic) applied at a rate of 65.1 g a.i. in 1.86 L/ha [authors also refer to this dose as 70g a.i./ha equivalent to 0.06 lb/acre] Two applications tebufenozide (Mimic) at rate of 33.4-35.4 g a.i in 1.91-2.02 L/ha to [authors also refer to this dose as 35 g a.i./ha equivalent to 0.03 lb/acre]	eastern hemlock looper	<u>One higher dose application</u>: • 9/10 plots showed reduction of loopers. • 9-11 days post-treatment, 3-93% reduction. • 3 weeks post-treatment 8-100% reduction. • Pupal populations reduced 8-99% • Defoliation of year-old foliage 10-51% (control plots 35-65%) and current-year foliage 0-16% (control plots 15-39%). <u>Two lower dose applications</u>: • 9-11 days post-treatment, in general, >50 % reduction. • 3 weeks post-treatment, in general >60% reduction. • Pupal populations reduced 76-100% • Defoliation of year-old foliage reduced 1-33% (control plots 35-65%) and current-year foliage reduced 0-8% (control plots 15-39%). For both treatments, plots with poor efficacy were associated with low foliar deposition, with deposits <1.5 μg/g foliage (deposition measured for each plot) associated with ineffective control.	

Appendix 7: Toxicity of tebufenozide to fish.

Species	Exposure	Response	Reference
ACUTE			
Bluegill sunfish (*Lepomis macrochirus*), mean wt = 0.32 g, mean length = 24 mm, juveniles, 10 fish/dose group	nominal concentrations of 0, 0.5, 1.0, 2.0, 5.0, 10.0, 25.0, or 100 mg ai/L; mean measured concentrations of 0, 0.39, 0.90, 2.2, 4.0, 5.7, 9.4, or 18 mg ai/L (ranging from 18-100% of nominal concentrations) for 96 hours under static conditions	No toxicity observed at concentrations ≤0.39 mg ai/L 96 hr LC_{50} = 3.0 mg ai/L (95% CI = 2.2 and 4.0 mg ai/L) NOEC = 0.39 mg ai/L	Graves and Smith 1992b MRID 42436239
Rainbow trout (*Oncorhynchus mykiss*), juveniles, mean wet wgt = 0.39 g, mean standard length = 28mm, 2 replicates of 10 per dose group	nominal concentrations of 0, 0.5, 1, 2, 5, 10, 25 or 100 mg ai/L; mean measured concentrations of 0, 0.42, 0.84, 1.9, 4.7, 7.2, 10, or 17 mg ai/L for 96 hours under static conditions	96 hr LC_{50} = 5.7 mg ai/L (95% CI = 4.7 and 6.5 mg ai/L) NOEC = 1.9 mg ai/L no signs of toxicity at concentrations ≤1.9 mg ai/L; mortality data from the highest dose group was not used to calculate the LC_{50} values.	Graves and Smith 1992c MRID 42436240

Appendix 7: Toxicity of tebufenozide to fish.

Species	Exposure	Response	Reference
LONGER-TERM			
Fathead minnow (*Pimephales promelas*), newly fertilized eggs (<24 hours after fertilization) used to initiate full life cycle study, 4 replicates of 25 animals per dose group.	mean measured concentrations of 0, 0.048, 0.090, 0.18, 0.35, or 0.72 mg ai/L (ranging from 92-100%) of nominal concentrations (0.048, 0.095, 0.19, 0.38, or 0.75 mg ai/L) under flow-through conditions. Both untreated and vehicle (acetone) control groups were assayed.	No effects on egg hatchability, parental generation growth, reproductive activity, or F_1 generation survival at any test concentration. Parental generation **survival was significantly decreased** at the two highest dose levels (0.35 and 0.72 mg ai/L): mean survival = **66% at 0.35 mg ai/L** (mortality = 22/25, 20/25, 7/25, and 17/25 in replicate groups A,B,C, and D, respectively) and **33% at 0.72 mg ai/L** (mortality = 9/25, 17/25, 3/25, and 4/25 in replicate groups A,B,C, and D, respectively).	Rhodes and Leak 1996 MRID 44221901 Reinert et al. 1999 MRID 44831501
Fathead minnows (*Pimephales promelas*), 30 days post hatch	nominal concentrations: 0, 0.063, 0.13, 0.25, 0.50, or 1.0 mg ai/L; mean measured concentrations: 0, 0.084, 0.14, 0.22, 0.36, or 0.71 mg ai/L by continuous exposure for 35 days. Both untreated and solvent controls were used.	The study and the supplement report no adverse effects on organism survival at hatch, larval survival and larval length and weight at any concentration levels. The U.S. EPA has classified the 0.71 mg/L concentration as an effect level based on decreased survival (88%) relative to survival in the solvent control (98%).	Bettancourt 1992 MRID 42436242 Surprenant 1994 MRID 43145701 (Supplement)

Appendix 8: Toxicity of tebufenozide to aquatic invertebrates and algae.

Plant or Animal	Exposure	Response	Reference
		Aquatic Invertebrates	
ACUTE			
Cladoceran (*Daphnia magna*), neonates (<24-hours old), 2 replicates of 10 each per dose group	nominal test concentrations: 0, 0.25, 0.50, 1.0, 2.5, 5.0, 10, or 100 mg ai/L; mean measured concentrations: 0, 0.22, 0.50, 0.82, 1.8, 4.7, 6.4, or 35 mg ai/L for 48 hours under static conditions	48-hour LC_{50} = 3.8 mg ai/L (95% CI = 2.9 and 5.1 mg ai/L) NOEC = 0.82 mg ai/L no signs of toxicity at concentrations ≤0.82 mg ai/L; values >1.8 ai/L were considered to be above the functional water solubility of the test substance.	Graves and Smith 1992a MRID 42436241
Northern lobsters (*Homarus americanus*), juveniles, 50-80 mm long	1.0, 10, or 100 µg ai/L Confirm 2F for 96 hours under static conditions	No adverse effects on survival and behavior.	Dionne 1998 MRID 44945701
Midge larvae (*Chironomus riparius*), 20 larvae (2 replicates of 10 animal each)	0, 0.05, 0.1, 0.2, 0.4, or 0.8 mg ai/L for 96 hours under static conditions Both untreated and solvent controls (acetone 0.10 mL/L).	96-hour aqueous LC_{50} = 0.30 mg ai/L (95%CI = 0.23-0.40 mg ai/L 96-hour NOEC = 0.12 mg ai/L both values based on mean measured concentrations.	van der Kolk 1997 MRID 44198301

Appendix 8: Toxicity of tebufenozide to aquatic invertebrates and algae.

Plant or Animal	Exposure	Response	Reference
Aquatic Invertebrates (continued)			
LONGER-TERM			
Daphnia magna, 10 per replicate vessel	Continuous exposure to 16, 29, 59, 120, or 240 µg ai/L for 21 days under flow-through conditions.	**Mortality**: at 21 days, average mean survival at 240 µg ai/L group= 50%, significantly less ($p<0.05$), than controls (96%); survival in lower dose groups ranged from 93-100%.	McNamara 1991 MRID 42436243

Additional Notes on McNamara 1991:
Reproduction: at 120 µg ai/L, statistically significant decrease ($p \leq 0.05$) in average rate of offspring/female (n=143), compared with controls (n=188); at lower concentrations, rate of offspring/females ranged from 226 to 239, which is statistically comparable to control.

Growth: at 120 µg ai/L, statistically significant decrease ($p \leq 0.05$) in mean total body length (5.0 mm), compared with controls (5.4 mm); at lower concentrations, mean total body length ranged from 5.3 to 5.5, which is statistically comparable to controls;

at 59 and 120 µg ai/L, statistically significant decrease ($p \leq 0.05$) in mean dry weight (1.3 and 1.6 mg, respectively), compared with controls (1.9 mg); at lower concentrations, mean dry weight ranged from 1.9 to 2.0, which is statistically comparable to controls;

LOEC = 59 µg ai/L; NOEL = 29 µg ai/L
21-day EC_{50} = 250 µg ai/L (lower 95% confidence interval of 120 µg ai/L)

Midge larvae (*Chironomus riparius*), 2- to 3-days old, 4 replicates per dose group	0, 0.0035, 0.0053, 0.0079, 0.012, 0.018, 0.027, 0.040, 0.060,0.090, or 0.135 mg ai/L for 28 days Both untreated and solvent controls (acetone 0.10 mL/L).	No effect on development rate of midge at any concentration; at ≥0.040 no midge emerged, which precluded the calculation of a development rate; at 0.0053, there was a statistically significant ($p \leq 0.05$) decrease in emergence rate; NOEC = 0.0035.	van der Kolk 1997 MRID 44198301

Appendix 8: Toxicity of tebufenozide to aquatic invertebrates and algae.

Plant or Animal	Exposure	Response	Reference
		Aquatic Algae	
Freshwater green alga (*Scenedesmus subspicatus*)	0.046, 0.077, 0.15, 0.25, or 0.66 mg ai/L (63-89% of nominal concentration) for 96 hours. Both untreated and solvent controls (acetone 0.10 mL/L).	**Cell density:** at 0.077, 0.15, 0.25, and 0.66 mg ai/L, respective cell densities averaged 81, 58, 52, and 37 x 10^4 cells/mL and were statistically reduced compared with pooled control cultures (114 x 10^4 cells/mL); at the lowest treatment level, cell density was statistically similar to that of controls). **Growth rate**: at 0.15, 0.25, and 0.66 mg ai/L, the 72-96 hr growth rates were 0.259, 0.310, and 0.004 $days^{-1}$, respectively and were statistically reduced compared with the growth rate of pooled controls (0.594 $days^{-1}$) NOEC for 72-96 hr growth rate = 0.077 mg ai/L. The 96 hr EC_{50} = 0.21 mg ai/L (95% confidence limit = 0.071-0.63 mg ai/L)	Hoberg 1992a MRID 42629501
Freshwater green alga (*Selenastrum capricornutum*) replicate 50 mL cultures (3 per treatment levels)	Nominal concentration of 0.80 mg ai/L for 120 hours	Empirically estimated EC_{50} >0.64 mg ai/L NOEC (based on reduced cell density) = 0.64 ai/L Treated algal culture reduced in density by 9.1% compared with controls	Hoberg. 1992b MRID 42436245 Reinert. 1993b MRID 42822201

Appendix K
DDVP (Dichlorvos) Risk Assessment

Figure K-1. A sprayer unit mounted on a Model A Ford truck was used for gypsy moth control.

SERA TR 04-43-05-05c

Control/Eradication Agents for the Gypsy Moth -
Human Health and Ecological Risk Assessment for
DDVP (Dichlorvos)
FINAL REPORT

Prepared for:

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AEL	adverse-effect level
ACGIH	American Conference of Governmental Industrial Hygienists
AChE	acetylcholinesterase
a.i.	active ingredient
BCF	bioconcentration factor
bw	body weight
CBI	confidential business information
ChE	pseudo-cholinesterase
CI	confidence interval
cm	centimeter
CNS	central nervous system
DAA	days after application
DAT	days after treatment
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
ExToxNet	Extension Toxicology Network
F	female
FH	Forest Health
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FOIA	Freedom of Information Act
FQPA	Food Quality Protection Act
g	gram
ha	hectare
HQ	hazard quotient
IAA	indole-3-acetic acid
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
k_a	absorption coefficient
k_e	elimination coefficient
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
$K_{o/w}$	octanol-water partition coefficient
K_p	skin permeability coefficient
L	liter
lb	pound
LC_{50}	lethal concentration, 50% kill
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level

LOC	level of concern
m	meter
M	male
MCL	mononuclear cell carcinoma
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
mM	millimole
MRID	Master Record Identification Number
MSDS	material safety data sheet
MW	molecular weight
NCAP	Northwest Coalition for Alternatives to Pesticides
NCI	National Cancer Institute
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
OPIDN	organophosphate-induced delayed neurotoxicity
OPP	Office of Pesticide Programs
OPPTS	Office of Pesticide Planning and Toxic Substances
OSHA	Occupational Safety and Health Administration
ppm	parts per million
PVC	polyvinyl chloride
RBC	red blood cell
RED	re-registration eligibility decision
RfD	reference dose
SERA	Syracuse Environmental Research Associates
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SRC	Syracuse Research Corporation
STS	Slow the Spread
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
WCR	water contamination rate
WHO	World Health Organization
µ	micron

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

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

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

CONVERSION OF SCIENTIFIC NOTATION

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

EXECUTIVE SUMMARY

OVERVIEW

The USDA uses DDVP in its program to manage the gypsy moth. The primary use of DDVP is as a component in the pheromone baited milk carton style traps that are used primarily for surveying and monitoring gypsy moth populations. Because of this a very limited use in USDA programs, the potential for exposures to humans or nontarget ecological species is extremely limited. Because of this limited use and limited potential for exposure, this risk assessment focuses on the information that has the greatest impact on potential hazard rather than a summary of all of the available information that is available on DDVP and this risk assessment utilizes several detailed reviews conducted by agencies responsible for assessing chemical risks

PROGRAM DESCRIPTION

In USDA programs for the control of the gypsy moth, DDVP is used only in a 1" x 4" inch polyvinyl chloride (PVC) strip that contains 590 mg of DDVP. These strips are used to kill insects that are attracted to and enter milk carton style traps baited with the gypsy moth pheromone. Typically milk carton traps are deployed in widely spaced grids (inter-trap distances ranging from 500 m to 7 km) to survey for the presence of gypsy moth populations in the STS or eradication areas. Only rarely are milk carton traps deployed in mass trapping grids to control isolated infestations. When used in mass trapping control efforts, milk carton traps are deployed in tightly spaced grids (inter-trap distance of 20 to 30 meters). Mass trapping is a rarely used eradication tactic that targets low-density infestations (<10 egg masses per acre) occupying relatively small areas (<100 acres) .

HUMAN HEALTH RISK ASSESSMENT

Hazard Identification **–** DDVP is an organophosphorus insecticide that works by inhibiting cholinesterase. DDVP has been used since the early 1960's and has been the subject of many toxicity studies and review articles. Information is available on a number of case reports of accidental and suicidal exposures as well as human monitoring data from normal use. The toxicity of DDVP has been adequately evaluated using laboratory animals, although not all of these studies are available in the open literature.

DDVP is readily absorbed into the body of mammals via all routes of exposure, where it is rapidly metabolized and eliminated. In general, the systemic effects observed after oral, inhalation, or dermal exposure of humans or laboratory animals to DDVP result from the inhibition of acetylcholinesterase. Inhibition of this enzyme in mammalian systems produces a variety of systemic effects, including salivation, urination, lacrimation, convulsions, increased bronchial secretions, respiratory depression, and even death. The nature and magnitude of the toxic effects produced by a given exposure to DDVP by any route are directly related to the dose and rate at which the exposure occurs. In the case of the USDA programs for the management of the gypsy moth, the use of milk carton traps containing Vaportape II (slow-release of DDVP from PVC strips) essentially precludes rapid exposures to high doses of DDVP.

Short-term animal studies have shown that oral exposures to doses below about 0.5 mg/kg-day (or inhalation exposures to 1–2 mg/m³) do not result in meaningful reductions in cholinesterase activity. Experiments in laboratory mammals that were exposed to DDVP during pregnancy (by oral or inhalation route) did not show any effect on fertility or health of the offspring, even at levels that produced maternal toxicity. The latest evaluation of data from assays for carcinogenicity and genetic toxicity classify DDVP as a "suggestive" carcinogen and determined that a quantitative assessment of cancer risk is not applicable. The literature contains some data suggesting that contact dermatitis (as well as cross-sensitization to other pesticides) may occur; although, this appears to be an infrequent occurrence in the general population.

Exposure Assessment – Under normal conditions, exposure to both workers and members of the general public should be negligible. Workers will handle DDVP strips only during the assembly of milk cartoon traps. If workers wear gloves and assemble the traps outdoors or in very well ventilated rooms, both inhalation and dermal exposures should be negligible. Inhalation exposure to DDVP during transport of the traps should also be negligible if the traps are not transported inside of the passenger compartments of vehicles. Worker exposures will also be limited in most programs because foil wrapping in which the strip is distributed will not be removed until after the trap is transported to the field. Milk carton traps will generally be placed about four feet above the ground (Leonard 2004) and exposure of members of the general public to DDVP contained in the milk carton traps should also be negligible except in the case of intensional tampering.

Notwithstanding the above assertions, exposure assessments are developed for workers who do not use gloves in the assembly of the milk carton traps and who assemble the traps indoors and transport the traps in the passenger compartments of vehicles. All of these exposure scenarios should be considered atypical and some are extreme. The intent is to illustrate the consequences of mishandling or imprudent handling. During assembly, the central estimate of dermal exposures in workers not wearing gloves leads to an absorbed dose of about 0.0008 mg/kg with a range of about 0.0003 mg/kg to 0.004 mg/kg. Inhalation exposures in workers may be highly variable depending on the ventilation rates in an enclosed space and the number of traps that are handled. Based on the handling and transport of 75 traps, inhalation exposures could reach up to about 0.6 mg/m^3 in an enclosed and unventilated room and up to about 1.8 mg/m^3 in the passenger compartment of a vehicle. These exposure assessments are based on several site and situation specific assumptions which are intended to reflect plausible upper bounds of exposures.

Exposure assessments are also developed for children who might come in contact with an accidentally discarded or misplaced DDVP strip. Estimated dermal doses are much higher than those for workers: a central estimate of about 0.02 mg/kg with a range of 0.003 mg/kg to 0.1 mg/kg. Oral exposures from a small child sucking on the pest strip are about a factor of 10 higher than dermal exposures: a central value of about 0.2 mg/kg with a range of 0.04 mg/kg to 0.6 mg/kg.

Under normal circumstances, the use of DDVP in PVC strips is not likely to result in contamination of water or other materials that might be consumed by members of the general public. Nonetheless, an exposure assessment is developed for the accidental contamination of a small pond by a pest strip. In this scenario, dose estimates range from about 0.000003 mg/kg to 0.00007 mg/kg with a central estimate of about 0.00001 mg/kg.

Dose-Response Assessment – The extensive toxicology data base has been evaluated by a number of governmental organizations including the U.S. EPA, the Agency for Toxic Substances and Disease Registry (ATSDR), National Institute for Occupational Safety and Health (NIOSH), Occupational Safety and Health Administration, and the World Health Organization. Following the approach taken in most USDA risk assessments, these sources are used for selecting levels of acceptable exposure. Because all of the scenarios considered in this risk assessment involve only acute exposures, only acute exposure criteria are considered.

For both oral and dermal exposures, the acute RfD established by the U.S. EPA, 0.0017 mg/kg, is used for the risk characterization. This is based on an acute oral NOAEL of 0.5 mg/kg from a study in rats with the application of an uncertainty factor of 300. Acute exposure criteria proposed by other groups are comparable to but somewhat higher than the acute RfD. Because some of the accidental acute exposures may substantially exceed the acute RfD, some attempt is made to characterize the consequences of high oral exposures. A human NOAEL of 1 mg/kg for AChE inhibition has been identified. While this NOAEL is not used to modify the acute RfD, it can be used to assess plausible consequences of exceeding the RfD. The human data on DDVP, although extensive, are not sufficient to identify a minimal lethal dose. For the current risk assessment, the lowest reported lethal dose (16 mg/kg) is used to assess the plausibility of observing serious adverse effects in cases of accidental over-exposure to DDVP.

A number of inhalation criteria for DDVP are available. Since potentially significant inhalation exposures are likely only in workers, the occupational exposure criteria of 0.1 mg/m^3 proposed by American Conference of Governmental Industrial Hygienists is used. This value is a factor of 10 below the occupational criteria proposed by NIOSH and OSHA.

Risk Characterization – In most cases, exposures to both workers and members of the general public should be negligible. If workers take prudent steps to limit both dermal and inhalation exposures, the likelihood of exposures to DDVP reaching a level of concern appears to be very low. Similarly, members of the general public should not be exposed to substantial amounts of DDVP. The DDVP is contained within a PVC strip to insure that the active ingredient is slowly released over a long period of time. The strip, in turn, is placed within a trap and the trap is placed so that the that will not be accessed except in the case of intentional tampering or trap monitoring.

Nonetheless, this risk assessment develops exposure scenarios for both workers and members of the general pubic that are intended to illustrate the potential effects of mishandling or tampering with DDVP strips. For workers, the greatest risks are associated with inhalation exposures from

assembling the traps in enclosed and poorly ventilated spaces or transporting the traps in the passenger compartments of vehicles. These risks can be readily avoided. Dermal exposures can also lead to lesser but sill undesirable levels of exposure. For members of the general public, all of the exposure scenarios are accidental and some are extreme. The most likely of these is the accidental contamination of a small body of water. This scenario leads to exposures that are below the level of concern by a factor of about 25. If a child were to come into contact with a DDVP strip, however, both dermal and oral exposures could substantially exceed a level of concern. While such exposures should clearly be avoided, it does not seem likely that frank signs of toxicity would be observed. This is consistent with human experience in the use of DDVP resin strips.

ECOLOGICAL RISK ASSESSMENT

Hazard Identification – The available data suggest that invertebrates are more sensitive to DDVP than other organisms. For example, the oral LD_{50} in honey bees is 0.29 µg/g bee, and the topical LD_{50} is 0.65 µg/g bee. DDVP is also toxic to birds with an oral LD_{50} value of < 10 mg/kg for the most sensitive species. Short-term repeat dose studies in mammals found that oral exposures to doses below about 0.5 mg/kg-day or inhalation exposures to 1–2 mg/m³ generally do not result in adverse effects.

Aquatic animals are also sensitive to DDVP and, as with terrestrial animals, invertebrates may be more sensitive than vertebrates. The lowest reported LC_{50} value in fish is approximately 0.2 mg/L. Some aquatic invertebrates are much more sensitive to DDVP than fish. For daphnids, the most sensitive group of invertebrate species, reported EC_{50} values range from 0.00007 mg/L to 0.00028 mg/L.

The majority of the toxicity data in ecological receptors is limited to free DDVP, rather than a slow-release formulation such as the Vaportape II product used in USDA programs for control of the gypsy moth. Hence, the toxicity values reported for indicator species will likely be conservative (i.e., suggest greater toxicity) as compared to Vaportape II. U.S. EPA has assessed the ecological effects of DDVP; however, the exposures assessed by U.S. EPA are not specific to formulations where DDVP is encapsulated in PVC resin. In general, aside from those organisms that enter the milk carton trap or those that remove the PVC strip form the trap, toxicity resulting from exposure of ecological receptors to DDVP in Vaportape II milk carton traps is not likely.

Exposure Assessment – As in the human health risk assessment, exposure of terrestrial mammals to DDVP from the VaporTape strips used in milk carton traps is likely to be negligible under most circumstances. Nonetheless, it is conceivable that some mammals such as racoons or bears could easily access and tamper with the milk carton trap. Depending on the proportion of the DDVP strip that is consumed, doses (as DDVP in the PVC strip) are estimated to range from 10.5 mg/kg (10% of strip) to 105 mg/kg (100% of strip) and the central estimate is taken as 31.6 mg/kg (30% of strip). In addition, contamination of water with a pest strip is plausible, although probably rare, and is considered in a manner similar to the corresponding scenario in the human health risk assessment (Section 3.2.3.4). This scenario is based on the consumption of

contaminated water by a small mammal and the dose to the animal is estimated at about 0.00003 mg/kg with a range of 0.000009 mg/kg to 0.00009 mg/kg. Other exposure scenarios for terrestrial vertebrates, while possible, seem far less plausible and are not considered quantitatively. No quantitative exposure assessments for terrestrial invertebrates are developed because the milk carton trap will attract only male gypsy moths. Nontarget insects that incidentally enter the trap are likely to be killed by exposure to the DDVP vapor. Exposures to aquatic species are based on the same water concentrations used for terrestrial species: 0.000177 mg/L with a range of 0.000059 mg/L to 0.00059 mg/L.

Dose-Response Assessment – Given the limited nature of the use of DDVP in programs to control the gypsy moth and consequent limited number of exposure assessments, the dose-response assessment for DDVP is relatively simple. For terrestrial mammals, a value of 240 mg/kg from a study using DDVP in a PVC formulation is used for direct exposure to the DDVP-PVC strip – i.e., a raccoon tampering with a milk carton trap and consuming all or part of the DDVP strip. At the dose of 240 mg/kg, no mortality or frank signs of AChE inhibition were observed. For the contaminated water scenario, the NOAEL of 0.5 mg/kg from a study involving exposure to free or unformulated DDVP is used. This NOAEL is from the study that forms the basis for the acute RfD used in the human health risk assessment. Although DDVP is classified as highly toxic to fish, the estimated levels of acute exposure for fish are far below the 30-day NOEC of 0.03 mg/L. Thus, this value is used for all fish and no attempt is made to consider differences in sensitivity among fish. A somewhat different approach is taken with aquatic invertebrates, some of which are more sensitive to DDVP than fish by a factor of over 2500. Risks to sensitive species of aquatic invertebrates – i.e., daphnids and other small arthropods – are characterized based on the lowest reported LC_{50} value, 0.00007 mg/L from a 48-hour bioassay in *Daphnia pulex*. Some other groups of aquatic invertebrates, such as snails, appear to be much less sensitive than small arthropods. Risks to such tolerant species are based on a LC_{50} value of 21 mg/L in a freshwater snail.

Risk Characterization – As with the human health risk assessment, it is anticipated that typical exposures and consequent risks to nontarget species should be negligible. As with the human health risk assessment, it is anticipated that typical exposures and consequent risks to most nontarget species should be negligible. The containment of the DDVP within a slow release PVC strip combined with the target specific nature of pheromone baited traps should reduce the risks of inadvertent effects in non-target species. Other insects and arthropods that may inadvertently enter the trap will probably be killed by DDVP vapor. While such inadvertent contact may occur, it is not likely to impact substantial numbers of nontarget insects or arthropods.

Because of the limited use of DDVP, a relatively small number of exposure scenarios – all of which might be considered accidental or incidental – are developed. For terrestrial mammals, contact with the pest strip could occur by an animal directly tampering with a trap or by an animal consuming water that had been accidentally contaminated with a DDVP strip. Adverse effects would not be expected in either case. In the case of accidental contamination of a small

body of water with a DDVP strip, concentrations of DDVP in the water would be below the level of concern for fish by factors of about 50 to 500. Some aquatic invertebrates, however, might be affected. For the most sensitive species of aquatic invertebrates – i.e., small aquatic arthropods such as daphnids – exposures could substantially exceed laboratory LC_{50} values by factors of up to about 8. Exposures to tolerant aquatic invertebrates – such as snails – would be below a level of concern by a substantial margin – i.e., factors of about 30,000 to 300,000.

The exposure assessments that serve as the bases for these risk characterizations are highly dependent on specific conditions – i.e., how much DDVP was in the strip at the time that the contamination occurred and the size of the body of water that was contaminated. Because the hydrolysis of DDVP in water is rapid, the estimates of adverse effects in some aquatic invertebrates would probably apply only to a very limited area near the pest strip rather than to the larger area of the body of water that is contaminated.

1. INTRODUCTION

The USDA uses DDVP in its program to manage the gypsy moth. The primary use of DDVP is as a component in the pheromone baited milk carton style traps that are used primarily for surveying and monitoring gypsy moth populations. This document is an update to a risk assessment prepared in 1995 (USDA 1995a,b) and provides risk assessments for human-health effects and ecological effects to support an assessment of the environmental consequences of these uses.

This document has four chapters, including the introduction, program description, risk assessment for human health effects, and risk assessment for ecological effects or effects on wildlife species. Each of the two risk assessment chapters has four major sections, including an identification of the hazards associated with DDVP, an assessment of potential exposure to the product, an assessment of the dose-response relationships, and a characterization of the risks associated with plausible levels of exposure. These are the basic steps recommended by the National Research Council of the National Academy of Sciences (NRC 1983) for conducting and organizing risk assessments.

Although this is a technical support document and addresses some specialized technical areas, an effort was made to ensure that the document can be understood by individuals who do not have specialized training in the chemical and biological sciences. Certain technical concepts, methods, and terms common to all parts of the risk assessment are described in plain language in a separate document (SERA 2001).

The human health and ecological risk assessments presented in this document are not, and are not intended to be, comprehensive summaries of all of the available information. This is particularly true for DDVP used in gypsy moth programs. There is an extremely large and relatively complex database of literature on DDVP. For example, TOXLINE, one of several commonly used commercial databases containing information on toxic chemicals, has over 14,000 citations on DDVP. DDVP, however, has a very limited use in USDA gypsy moth programs (Section 2) and the potential for exposures to humans (Section 3.2) or nontarget ecological species (Section 4.2) is extremely limited. Because of this limited use and limited potential for exposure, this risk assessment focuses on the information that has the greatest impact on potential hazard rather than a summary of all of the available information that is available on DDVP and this risk assessment utilizes several detailed reviews conducted by agencies responsible for assessing chemical risks (e.g., ATSDR 1997; U.S. EPA 1999a, 2000a,b; WHO 1988, 1989).

This risk assessment involves numerous calculations. Many of the calculations are relatively simple and the very simple calculations are included in the body of the document. Some of the calculations, however, are complex. For the more complex calculations, worksheets are included as an attachment to the risk assessment. The worksheets provide the detail for the estimates cited in the body of the document. The worksheets for DDVP are contained in an EXCEL workbook

and are included as Supplement 1 to this risk assessment and general documentation for the use of these worksheets is given in SERA (2004).

The USDA will update this and other similar risk assessments on a periodic basis and welcomes input from the general public on the selection of studies included in the risk assessment. This input is helpful, however, only if recommendations for including additional studies specify why and/or how the new or not previously included information would be likely to alter the conclusions reached in the risk assessments.

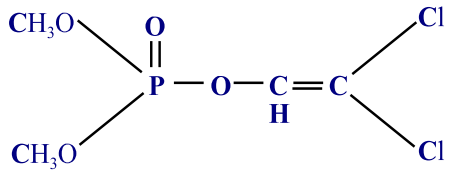
2. PROGRAM DESCRIPTION

2.1. OVERVIEW

DDVP is an organophosphate insecticide that acts by inhibiting acetylcholinesterase, an enzyme that is very important in the nervous system of all vertebrates and many invertebrates including all arthropods. Thus, DDVP is not specific to the gypsy moth or other insects. In USDA programs for the control of the gypsy moth, DDVP is used only in a 1" x 4" inch polyvinyl chloride (PVC) strip that contains 590 mg of DDVP. These strips are used to kill insects that are attracted to and enter milk carton style traps baited with the gypsy moth pheromone. Typically milk carton traps are deployed in widely spaced grids (inter-trap distances ranging from 500 m to 7 km) to survey for the presence of gypsy moth populations in the STS or eradication areas. Only rarely are milk carton traps deployed in mass trapping grids to control isolated infestations. When used in mass trapping control efforts, milk carton traps are deployed in tightly spaced grids (inter-trap distance of 20 to 30 meters). Mass trapping is a rarely used eradication tactic that targets low-density infestations (<10 egg masses per acre) occupying relatively small areas (<100 acres) .

2.2. CHEMICAL DESCRIPTION AND COMMERCIAL FORMULATIONS

DDVP is the common name for O,O-dimethyl O-(2,2-dichlorovinyl) phosphate:



Other synonyms for DDVP as well as selected chemical and physical properties of DDVP are summarized in Table 2-1.

DDVP is a contact and stomach organophosphate insecticide (Gallo and Lawryk 1991, IARC 1991). As detailed further in the human health risk assessment (Section 3) and the ecological risk assessment (Section 4), DDVP acts by inhibiting acetylcholinesterase, an enzyme that is very important in the nervous system of all vertebrates (including humans) and most other animals including all arthropods.

DDVP is currently undergoing reregistration (http://www.epa.gov/pesticides/op/ddvp.htm; Mennear 1998) and is being considered in the U.S. EPA's cumulative risk assessment of organophosphates (http://www.eps.gov/pesticides).

Various DDVP pest strips for residential or industrial use have been registered with the U.S. EPA and are manufactured by AMVAC Chemical Corporation, Loveland Industries, and Spectrum Group (http://www.cdpr.ca.gov/docs/pressrls/ddvp.htm). However, the only strip used by the USDA in gypsy moth programs is the Vaportape II strip provided by Hercon Environmental Corp, Emigsville, PA (Hercon 1993). A contract for the supply of these strips to

the USDA gypsy moth program was awarded to Hercon Environmental Corp on March 23, 1999 (www.fbodaily.com/cbd/archive/1999/03 (March)23/Mar-1999/87awdoo1.htm).

Vaportape II is distributed in packages of 50 strips, each of which comes in a protective pouch. Each strip consists of a 1" x 4" inch red, multi-layered polyvinyl chloride (PVC) strip containing 590 mg of DDVP. The average thickness of the strip is 67.5 mil with a range of 65–70 mil or 0.0675 inches with a range of 0.065–0.07 inches (Hercon 1994). Additional details concerning the composition of the strips have been disclosed to U.S. EPA (Health-Chem Corporation 19??; Herculite Products Incorporated 19??a,b; Starner 1993). Note that the 19?? designation indicates that the material is not dated and that the U.S. EPA cannot determine when the information was submitted. This is not uncommon for submissions that occurred in the early 1970's. The details of the information contained in these submissions are classified as CBI (confidential business Information) under Section 7(d) and Section (10) of FIFRA and this information cannot be specifically disclosed in this risk assessment.

The product label specifies that in addition to DDVP, each strip contains 0.75% compounds that are related to DDVP and 89.25% inerts (Hercon 2004). Further details are not provided on the label; nonetheless the impurities in commercial DDVP have been characterized (Gillett and others 1972a, IARC 1991). The impurities include: Dipterex (O,O-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate); O,O-dimethyl 2-chlorovinyl phosphate; O,O-dimethyl methylphosphonate; O,O,O-trimethyl phosphate; and trichloroacetaldehyde. These impurities are known to be or are likely to be toxic (Gillett and others 1972a, WHO 1989). These impurities are encompassed in the risk assessment because the dose-response assessment is based on studies that used commercial grade DDVP. Consequently, the results of these studies are directly applicable to the risk assessment for human health (Section 3) and ecological effects (Section 4).

2.3. APPLICATION METHODS

The Vaportape II strips are used as an insecticide in large capacity pheromone traps to monitor gypsy moth populations. DDVP is also used in a similar way in monitoring populations of the beet armyworm (Lopez 1998).

In order to minimize the ecological effects and human health effects of gypsy moth infestations, the USDA adopted various intervention strategies that are roughly categorized as suppression, eradication, and Slow the Spread (STS). Suppression efforts are conducted by the USDA Forest Service in areas of well established gypsy moth infestations to combat or interdict periodic gypsy moth population outbreaks. Eradication efforts are conducted by USDA/APHIS to completely eliminate gypsy moth populations in areas where new populations of the gypsy moth are found. Slow the spread, as the name implies, is a program to reduce the expansion of gypsy moth populations from areas of established populations to adjacent non-infested areas (Liebhold and McManus 1999). The STS project is the primary user of DDVP and milk carton traps. STS has purchased DDVP in the following amounts: 2002 - 540 packs (540x50 strips=27,000 strips); 2003 - 540 packs (27,000 strips); 2004 - 500 packs (25,000 strips) (Leonard 2004).

As in the previous gypsy moth programs, a Vaportape II strip is contained in the milk carton trap together with together with a slow release dispenser containing disparlure, the gypsy moth pheromone. The milk carton traps containing the strips are placed in selected areas to monitor gypsy moth infestations. When used in eradication efforts for mass trapping, milk carton traps are typically used only in low density infestations – i.e., 10 egg masses per acre or less. In addition, because of the labor involved in mass trapping, this method is applied to relatively small areas – i.e., about 100 acres or less (USDA 2001, p. 1-7 to 1-8).

As discussed in the exposure assessments for human health (Section 3.2) and ecological effects (Section 4.2), the nature of the exposures to humans and other nontarget species will typically be extremely small and it is unlikely that significant exposures will occur under normal circumstances. For workers, the nature of exposure to DDVP depends on program handling practices, which vary from state to state. In most cases, dermal and inhalation exposure will be minimal, provided that recommended work practices are followed. In some states, inhalation exposure will be minimal because strip installation takes place outdoors, at the trap placement site. In other states, traps may be assembled the day before placement. Even so, the workers are instructed to assemble the traps only in a well-ventilated area, and the traps are sealed in plastic bags after assembly and prior to transport. Dermal exposure is also likely to be minimal. In most states, workers are given plastic gloves and instructed to use them. In other states, workers are instructed to touch only the plastic wrapper in which the strip is shipped.

3. HUMAN HEALTH RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION

3.1.1. Overview

DDVP is an organophosphorus insecticide that works by inhibiting cholinesterase. DDVP has been used since the early 1960's and has been the subject of many toxicity studies and review articles. Information is available on a number of case reports of accidental and suicidal exposures as well as human monitoring data from normal use. The toxicity of DDVP has been adequately evaluated using laboratory animals, although not all of these studies are available in the open literature.

DDVP is readily absorbed into the body of mammals via all routes of exposure, where it is rapidly metabolized and eliminated. In general, the systemic effects observed after oral, inhalation, or dermal exposure of humans or laboratory animals to DDVP result from the inhibition of acetylcholinesterase. Inhibition of this enzyme in mammalian systems produces a variety of systemic effects, including salivation, urination, lacrimation, convulsions, increased bronchial secretions, respiratory depression, and even death. The nature and magnitude of the toxic effects produced by a given exposure to DDVP by any route are directly related to the dose and rate at which the exposure occurs. In the case of the USDA programs for the control of the gypsy moth, the use of milk carton traps containing Vaportape II (slow-release of DDVP from PVC strips) essentially precludes rapid exposures to high doses of DDVP.

Short-term animal studies have shown that oral exposures to doses below about 0.5 mg/kg-day (or inhalation exposures to 1–2 mg/m^3) do not result in meaningful reductions in cholinesterase activity. Experiments in laboratory mammals that were exposed to DDVP during pregnancy (by oral or inhalation route) did not show any effect on fertility or health of the offspring, even at levels that produced maternal toxicity. The latest evaluation of data from assays for carcinogenicity and genetic toxicity classify DDVP as a "suggestive" carcinogen and determined that a quantitative assessment of cancer risk is not applicable. The literature contains some data suggesting that contact dermatitis (as well as cross-sensitization to other pesticides) may occur; although, this appears to be an infrequent occurrence in the general population.

3.1.2. Mechanism of Action

The mechanism of action of DDVP in target organisms and its principal toxic effects in humans and animals result from inhibiting neural acetylcholinesterase (AChE). DDVP shares this mechanism of action with other organophosphate insecticides. A number of excellent reviews on the mechanism of action of the organophosphate insecticides are available in various texts (Wills 1972; Gallo and Lawryk 1991; Taylor 1996; Ecobichon 2001). The AChE enzyme is present at cholinergic synapses (spaces between the nerve cells) throughout the nervous systems, and it is responsible for hydrolyzing acetylcholine released from the pre-synaptic terminal. If this enzyme is inhibited, acetylcholine accumulates in the synapse, resulting in increased stimulation of the postsynaptic neuron and cholinergic overstimulation. The consequences of increased cholinergic activity in various organ systems are listed in Table 3-1. These classical symptoms of

organophosphate neurotoxicity increase in severity and rapidity of onset in a dose-dependent manner.

Acetylcholinesterase is also present in erythrocytes where it is known as erythrocyte or red blood cell acetylcholinesterase (RBC AChE). *In vitro* assays have found that the erythrocyte and neural forms of AChE are inhibited to roughly the same extent by exposure to DDVP (ATSDR 1997). Measurement of RBC AChE is used as a surrogate of the inhibition of neural AChE. One of the major diagnostic tools and measures of exposure to DDVP and other organophosphate insecticides is the determination of cholinesterase activity in various tissues, most often red blood cells and plasma (Ecobichon 2001; Gallo and Lawryk 1991; Murphy 1980). Plasma cholinesterase, sometimes referred to as pseudo-cholinesterase or ChE, is produced by the liver and differs from AChE in structure and substrates (ATSDR 1993). Although the normal physiological role of plasma ChE is not known, it is also inhibited by DDVP and is often used as a marker for exposure. Inhibition of RBC AChE is generally regarded as a more clinically significant index of organophosphate exposure, compared with inhibition of plasma ChE, as plasma ChE is inhibited by DDVP at lower levels of exposure than required to inhibit neural or erythrocyte AChE (ATSDR 1997).

3.1.3. Kinetics and Metabolism

DDVP is a small, lipid-soluble molecule (see Table 2-1) that is readily absorbed by passive diffusion through the lungs, gastrointestinal tract, or skin. Little information is available on the pulmonary absorption rate of DDVP, but it appears to be rapidly absorbed by the inhalation as well as oral and dermal routes of exposure. Due to the rapid degradation of DDVP by tissue esterases, particularly in the liver and the serum, measuring DDVP *in vivo* is difficult. Laws (1966) reported that DDVP is absorbed primarily by hepatic portal venous system after oral administration and is subject to first pass metabolism by the liver. Because of the difficulty in measuring DDVP *in vivo*, the rate of absorption is typically inferred from the time to onset of clinical signs of AChE inhibition (see Table 3-1). Determination of the tissue distribution of DDVP is also difficult to study because of rapid metabolism, but the data do not suggest preferential distribution or sequestration in any tissue (ATSDR 1997). A compartmental model has been proposed by Garcia-Repetto et al. (1995) to describe the toxicokinetics of DDVP following oral exposure. The model was composed of two compartments: central and peripheral. The central compartment was blood, and the peripheral compartment encompassed adipose, muscle, and liver.

3.1.3.1. Oral Absorption – Oral absorption of DDVP is rapid. Acute oral toxicity studies have demonstrated toxic effects from oral DDVP exposure within minutes. ATSDR (1997) noted that animal studies demonstrated lethality from single gavage doses of DDVP within 9 minutes for Swiss mice and 15–30 minutes in crossbred swine; signs of cholinergic toxicity (vomiting and diarrhea) were noted in greyhound dogs 7–15 minutes after receiving oral doses of DDVP in gelatin capsules. Based on a suicide case, Shimizu et al. (1996) have reported the tissue distribution of DDVP in humans following oral exposure. Tissue to blood ratios in this individual ranged from <1 for brain and liver to 28 for heart and 115 for the spleen. The authors

reported that the high-tissue concentrations in the heart and spleen were likely due to diffusion from the stomach to nearby organs (postmortem, the stomach contained approximately 250 mL of fluid equivalent to 300 g of DDVP). Studies in swine treated with DDVP-impregnated PVC pellets (veterinary use as anthelminthic) show that DDVP is absorbed from the PVC resin after oral exposure (Jacobs 1968, Potter et al. 1973).

3.1.3.2. Dermal Absorption – No studies have been found on the dermal absorption rate of DDVP in humans. As a small, lipid-soluble compound (see Section 2.2), DDVP would likely be rapidly absorbed through the skin. Dermal absorption in rats has been studied by Jeffcoat (1990). Groups of rats were dosed with ^{14}C-DDVP at 3.6, 36, and 360 µg/rat by applying the compound to the shaved back. The treated area was isolated with a protective cover for a 10-hour period. After 10 hours, the remaining DDVP was washed from the treated surface and animals were sacrificed over 24- to 102-hour periods. Based on the ^{14}C recovered from the rats, the amount penetrating the skin ranged from 21.9 to 30.1% with no substantial variation among dose groups. For this type of a study, first order dermal absorption coefficients (***k***) can be calculated as:

$$\boldsymbol{k} = -\ln(1-\boldsymbol{f})/\boldsymbol{t}$$

where ***f*** is the fraction absorbed and ***t*** is the duration of exposure. Based on absorption fractions of 0.219 to 0.301, the first-order dermal absorption rates can be calculated as 0.025 hour^{-1} [-ln(1-0.219)/10 hours] to 0.036 hour^{-1} [-ln(1-0.301)/10 hours]. These calculations are based on the cumulative amount of DDVP recovered from urine, feces, expired air, blood, carcass, and treated skin). Excluding treated skin, only 6.4 to 11.4% of the dose was actually absorbed. These correspond to first order dermal absorption rates of 0.0066 hour^{-1} [-ln(1-0.064)/10 hours] to 0.012 hour^{-1} [-ln(1-0.114)/10 hours] and these estimates are consistent with the dermal absorption rate selected by EPA (2000a) for occupational and residential exposures (11% in 10 hours of exposure).

3.1.3.3. Metabolism – As noted above, DDVP is rapidly degraded by tissue esterases, particularly in the liver and the serum. The products of the esterase-catalyzed degradation of DDVP are dimethyl phosphate and dichloroacetaldehyde. Dimethyl phosphate is excreted in the urine, while dichloroacetaldehyde can be reduced to dichloroethanol or dehalogenated to glyoxal, which enters 2-carbon metabolism. Dichloroethanol is either conjugated to glucuronic acid and excreted in the urine or dehalogenated and further metabolized. There is also evidence that DDVP can be demethylated in a glutathione-dependent reaction (WHO 1989, ATSDR 1997). The *in vitro* half-life of DDVP in human blood is about 10 minutes (Blair et al. 1975).

3.1.4. Acute Oral Toxicity

As described in Section 3.1.2, DDVP exposure can result in increased cholinergic activity in the nervous system, producing the classical symptoms of organophosphate poisoning (See Table 3-1). The life-threatening effects of acute exposure to DDVP are usually related to its cholinergic effects on the respiratory system (respiratory depression, bronchospasm, increased bronchial secretions, pulmonary edema, and muscle weakness). DDVP is moderately to highly

toxic by the oral route when administered in single doses to a variety of animal species, and several cases of acute DDVP poisoning in humans have reported in the literature. Some individuals have committed suicide by intentionally ingesting DDVP pesticide formulations (e.g., Shimizu et al. 1996). This study is discussed further in Section 3.3 (Dose-Response Assessment). In an attempted suicide, a 56-year old woman who ingested about 100 mg/kg DDVP survived following intensive care for 14 days (WHO 1989). Two workers who had skin exposure to a concentrated dichlorvos formulation, and failed to wash it off, died of poisoning. In addition, four patients suffering from severe poisoning from oral exposure to dichlorvos survived, although they later showed delayed neurotoxic effects (WHO 1989). Thus, although the possibility of neuropathy in humans cannot be excluded, it is likely to occur only after almost lethal oral doses (see also Section 3.1.6).

Oral LD_{50} values for experimental mammals range from 25 to 300 mg/kg (Jones et al. 1968, Gaines 1969, Muller 1970, Wagner and Johnson 1970). Signs of intoxication in these studies are consistent with cholinergic overstimulation, typically salivation, lacrimation, urination, defecation, tremors, convulsions, and death from respiratory failure.

EPA (2000a, p. 18) identified an unpublished neurotoxicity study in rats as the basis for establishing a risk level for acute oral exposure to unformulated DDVP – i.e., DDVP not in a PVC strip. In this study (Bast et al. 1997), Sprague Dawley rats (12/sex/dose) received a single oral dose of DDVP (97.8%) at doses of 0, 0.5, 35, or 70 mg/kg. Behavioral testing, including a functional observation battery and motor activity, was conducted pretest, 15 minutes after treatment, and on days 7 and 14 after exposure. Cholinesterase activity was not measured in any tissue. The acute NOAEL was 0.5 mg/kg and the LOAEL was 35 mg/kg based on neurological effects related to AChE inhibition.

The containment of DDVP in a slow-release vehicle, however, such as the PVC in the Vaportape II strips, will reduce the likelihood of acute toxic effects. The kinetics of DDVP release from PVC were investigated in a study in which DDVP was incorporated into PVC at 20% (w/w) (Slomka and Hine 1981). The PVC was extruded, cut into pellets, and encased in a hard gelatin capsule. The release of DDVP from the capsules was assayed *in vitro* using an artificial gastric fluid and *in vivo* in swine and humans. The release rates in the three assays were comparable; approximately 30% was released in the first 24 hours, and the subsequent release appeared to follow a first order function with a release rate of approximately 0.1 day^{-1}.

The effect of PVC encapsulation on the toxicity of DDVP has been quantified in parallel acute assays in young pigs (Stanton et al. 1979) using unformulated DDVP (undiluted technical grade administered in gelatin capsules) and DDVP in PVC resin (administered by gavage). For the technical grade liquid formulation, the LD_{50} was 157 (113–227) mg/kg. Signs of toxicity in these animals were consistent with the general signs of AChE inhibition (Table 3-1) and included decreased general activity, vomiting, poor coordination, and twitching. In the bioassay using the PVC formulation, no deaths occurred at any of the administered doses – i.e., 180 mg/kg, 240 mg/kg, 320 mg/kg or 1,000 mg/kg. Higher doses of the DDVP-PVC formulation could not be

administered because these doses produced vomiting. While not specified by Stanton et al. (1979), vomiting at doses >1,000 mg/kg may have been due to the physical stress associated with such a large gavage dose. Although no animals died, vomiting was observed at all DDVP-PVC doses. At the lowest dose, 180 mg/kg, vomiting with no other signs of AChE inhibition were observed. At the next higher dose, 240 mg/kg, no adverse effects are reported.

Stanton et al. (1979) also conducted 30-day assays using only the PVC formulation. Aside from alterations in cholinesterase activity, 30 consecutive days of exposure of young swine or gravid sows to doses as high as 25 mg/kg-day of the DDVP-PVC formulation produced no adverse effect on any physical or biochemical parameter measured. The authors suggest that the lack of serious adverse effects was related to the slow-release of DDVP from the PVC pellet (Stanton et al. 1979).

In an abstract, Singh et al. (1968) evaluated free DDVP (200 or 400 mg/day) or DDVP in V-13 pellet (800 mg/day; 9% DDVP, 91% inert [NOS]) in gravid sows. The DDVP, whether in free form or in the pellet, produced no adverse effects on the number of pigs born alive, number of pigs born dead, average birth weight, average number of pigs weaned at 35 days, or the average weanling weight. Minor gross signs of organophosphate poisoning (NOS) were observed only in the group receiving 400 mg/day free DDVP.

3.1.5. Subchronic or Chronic Systemic Toxic Effects

Subchronic and chronic toxicity bioassays have been conducted in several laboratory animal species (e.g., rats, mice, dogs, pigs, and monkeys), exploring the adverse effects of DDVP exposure by oral and inhalation routes of exposure. Generally, the toxic effects of DDVP exposure (regardless of route of administration) are due to the inhibition of AChE (Table 3-1). Consequently, plasma, erythrocyte, and brain cholinesterase activity are metrics of exposure and toxicity. Studies have demonstrated more sensitive neurological effects than cholinesterase inhibition; however, the toxicologic implications of these early biomarkers of exposure are uncertain. For example, the correlations between the relatively low level, chronic dichlorvos (DDVP) exposure and early electrophysiological changes (assessed by electrocorticogram, cortical evoked potentials, conduction velocity, and refractory periods of peripheral nerve) showed the electrophysiological parameters to be sensitive biomarkers of the exposure in humans (Desi et al. 1998).

In a long-term dietary study, rats fed diets containing DDVP for 2 years showed no signs of toxicity until the dietary exposures reached 2.5 mg/kg-day or more (WHO 1989). EPA (2000a) identified an unpublished dietary study in dogs (MRID No. 41593101 as summarized by U.S. EPA 1994) as the basis for establishing a risk level for chronic oral exposure. Groups of beagle dogs received DDVP orally in capsules at dose levels of 0, 0.1, 1.0, and 3.0 mg/kg/day for 52 weeks. The 0.1 mg/kg/day dose was lowered to 0.05 mg/kg/day on day 22 due to the inhibition of plasma ChE noted after 12 days (the magnitude of the reduction was 21.1% in males and 25.7% in females). After week 2, plasma ChE activity was only significantly reduced in males (39.1–59.2%) and females (41.0–56.7%) in the mid-dose group and in males (65.1–74.3%) and

females (61.1–74.2%) in the high-dose group at all other later time intervals. RBC AChE activity was reduced in males (23.6%) and females (50.1%) at week 6 in the low-dose group. The authors attributed this to a residual effect on RBC AChE of the earlier dose of 0.1 mg/kg/day, because much less inhibition was observed in this group after week 6. After week 6, RBC AChE activity was only significantly decreased in males (43.0–53.9%) and females (38.0–51.9%) in the mid-dose group and in males (81.2–86.9%) and females 79.2–82.5%) in the high-dose groups at all other later time intervals. Brain AChE activity was significantly reduced in males (22%) in the mid-dose group and in males (47%) and females (29%) in the high-dose group. The NOAEL and LOAEL selected by EPA (2000a) for chronic oral risk exposure are 0.05 and 0.1 mg/kg/day, respectively. These effect levels are based on plasma ChE and RBC AChE inhibition in male and female dogs as early as the first time point measure and brain AChE inhibition in male dogs.

3.1.6. Effects on Nervous System

A neurotoxicant is a chemical that disrupts the function of nerves, either by interacting with nerves directly or by interacting with supporting cells in the nervous system (Durkin and Diamond 2002). This definition of neurotoxicant 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). As discussed in Section 3.1.2, DDVP, like all organophosphate insecticides, is a direct-acting neurotoxicant. DDVP combines with and inhibits AChE. The biochemical basis for the toxic effects of DDVP is related to the normal function of AChE. In the cholinergic system, neural impulses are transmitted between nerve cells or between nerve cells and an effector cell (such as a muscle cell) by the acetylcholine. When the acetylcholine reaches a certain level, the receptor cell is stimulated. Normally, the acetylcholine is then rapidly degraded to inactive agents (acetic acid and choline) by AChE. When AChE activity is inhibited by organophosphate agents (such as DDVP), acetylcholine persists and continues to accumulate at the synapse (the space between the nerve cells). Initially, this accumulation causes continuous stimulation of the cholinergic system, which may be followed by paralysis because of nerve cell fatigue (ATSDR 1993).

The cholinergic effects of DDVP intoxication are well documented in studies involving humans, wildlife, and experimental mammals (Gillett et al. 1972a,b; IARC 1979, 1991; WHO 1989). DDVP also inhibits other cholinesterases and many other esterases outside of the nervous system and induces clinical signs of intoxication that are dependent upon the dose and duration of exposure (Table 3-1). In addition, some studies of lifetime exposure of rats to DDVP suggest that oral exposures to doses $\geq$0.97 mg/kg-day result in behavioral changes (Schultz et al. 1995, Institäoris et al. 1997).

RBC AChE activity follows a circadian oscillation in both mice and humans (Jian and Zhiying 1990). Furthermore, mortality in mice associated with exposure to DDVP is inversely related to the oscillation in AChE activity. These investigators report that DDVP interferes with the normal circadian rhythm of RBC AChE in mice and humans, although this interference is secondary to pronounced AChE inhibition.

The effect of DDVP on AChE activity in humans has been assayed by Gledhill (1997). In this study, DDVP was administered to 6 male volunteers as a single dose of 70 mg DDVP in a corn oil solution in a gelatin capsule. The body weights of 6 individuals ranged from 67 kg to 80 kg (Gledhill 1997) and thus the individual dose rates ranged from 0.70 to 1.04 mg/kg bw. No effect on AChE activity was observed and there were no signs or symptoms of cholinergic overstimulation.

Normal ChE activities can be highly variable among individuals. Consequently, interpreting differences between cholinesterase levels in exposed groups and control groups is more difficult than interpreting differences between individual ChE levels before and after exposure (ATSDR 1993). All of the human and animal studies on PVC-DDVP formulations report AChE levels using the method involving treated groups and control groups. For all of the human studies on DDVP (Cervoni et al. 1969; Pena-Chavarria et al. 1969; Hine and Slomka 1970; Slomka and Hine 1981), the interpretation is further complicated because ChE levels are reported as ranges of inhibition, rather than mean values with standard errors.

As discussed in the general literature and illustrated in the human studies on DDVP, inhibition of cholinesterase in plasma and blood is not necessarily associated with clinically significant adverse effects (Gage 1967; Wills 1972). ATSDR (1997) noted that the nervous system can accept a certain amount of acetylcholinesterase inhibition without overt toxic effects. In humans and animals, toxic signs are generally not seen until at least 20% of this enzyme (RBC AChE used as a marker) has been inhibited (ATSDR 1997). In a rat study, brain AChE after a 2-year inhalation exposure to DDVP was inhibited more than 90% compared to control animals (Blair et al. 1976), yet signs of cholinergic overstimulation were not observed. ATSDR (1997) suggests that the best predictor of toxicity is not necessarily the actual percentage inhibition of AChE, but rather how rapidly this inhibition has occurred. Rapid inhibition does not afford the nervous system time to adapt to AChE inhibition. This adaptation appears to involve desensitization and down regulation of muscarinic receptors (ATSDR 1997).

A significant characteristic of some organophosphate insecticides is that the reversibility of enzyme inhibition is slow (Murphy 1980). Relatively little information is available on the reversibility of inhibition due to DDVP. There is one case report indicating substantial inhibition of ChE, 36% of normal, in an individual exposed to DDVP 3 days before the assay of ChE activity (Bisby and Simpson 1975), and other data suggest that cholinesterase activity levels do not return to normal for several months (ATSDR 1997).

Exposure to some organophosphorus compounds cause delayed neuropathy in humans (also known as organophosphate-induced delayed neurotoxicity or OPIDN). Clinical manifestations include motor dysfunction, tingling in the extremities, and in some cases paralysis. These effects usually appear 7–14 days after exposure, when signs of cholinergic toxicity have resolved, and can persist for weeks or years (ATSDR 1997). The data concerning the potential for DDVP-induced OPIDN are inconsistent and controversial. Several studies that demonstrate that DDVP does not induce delayed neuropathy (WHO 1989), including a recent study in adult hens

(Abdelsalam 1999). On the other hand, very high doses of DDVP (doses in excess of the LD_{50}) produced clinical neuropathy when administered to hens (Johnson 1978, 1981). These data are consistent with human cases of poisoning where recovery was followed by delayed neurotoxicity (see Section 3.1.4) (WHO 1989). Subcutaneous doses of DDVP (single dose of 200 mg/kg or 6 mg/kg-day for 8 weeks) in rats led to motor deficit or biochemical and behavioral deficits (Sarin and Gill 2000, 1998, respectively). The potential for OPIDN in humans resulting from exposure to DDVP in PVC resin strips is unknown.

3.1.7. Effects on Immune System

Immunotoxicants are chemical agents that disrupt the function of the immune system. Two general types of effects, suppression and enhancement, may be seen and both of these are generally regarded as adverse. Agents that impair immune responses (*immune suppression*) enhance susceptibility to infectious diseases or cancer. Enhancement or *hyperreactivity* can give rise to *allergy* or hypersensitivity, in which the immune system of genetically predisposed individuals inappropriately responds to chemical or biological agents (e.g., plant pollen, cat dander, flour gluten) that pose no threat to other individuals or *autoimmunity*, in which the immune system produces antibodies to self components leading to destruction of the organ or tissue involved (Durkin and Diamond 2002).

Although the literature contains some evidence that organophosphate insecticides can impair immunological markers (Colosio et al. 1999), no human data are available to describe a dose-response relationship for the immunotoxic potential of DDVP. Animal studies suggest that exposure to DDVP may be associated with immunosuppression. Treating rabbits with oral doses of 0.31–2.5 mg/kg DDVP (2.5–20% of the LD_{50}) 5 days per week for 6 weeks resulted in inhibition of both humoral and cell-mediated immune response to *S. typhimurium* (Desi et al. 1978, 1980). Immunosuppression (suppressed IgM response at 48 hours) was also observed in mice treated with a single oral dose of 120 mg/kg DDVP (Casale et al. 1983). A decrease in relative spleen weight was also noted in this study; however, severe signs of DDVP neurotoxicity were noted and the authors stated that the immunosuppression observed in this study may have been related to toxic chemical stress. In addition, *in vitro* studies on cells from embryonic renal tissue of carp demonstrated a dose-related decrease in lymphocyte proliferation and myeloid cell respiratory burst activities, both of which indicate immunosuppression; however, no effects on antibody production were noted in an *in vivo* study of carp (Dunier et al. 1991). Bryant (1985) has associated the precipitation of preexisting asthma to small doses (NOS) of DDVP.

Aside from the few positive reports above, there is very little direct information on which to assess the immunotoxic potential of DDVP in humans. The extrapolation of the observed alterations in the immune system response of experimental animals to humans is uncertain, since the functional relevance of these deficits in humans is unknown. The immune system has a functional reserve and modifications in the immune response do not always correlate with a measurable health effect (Vial et al. 1996; Voccia et al. 1999).

The systemic toxicity of DDVP has been adequately examined in numerous acute, subchronic, and chronic bioassays. Although many of these studies did not focus on the immune system, changes in the immune system (which could potentially be manifest as increased susceptibility to infection among DDVP-exposed animals compared to controls) were not observed in any of the available long-term animal studies. In a three-generation study of Wistar rats, neurologic endpoints were found to be more sensitive markers of exposure than immunologic endpoints in all three generations (Institäoris et al. 1997).

3.1.8. Effects on Endocrine System

In terms of functional effects that have important public health implications, some of the effects on endocrine function would be expressed as diminished or abnormal reproductive performance. This issue is addressed specifically in the following section (Section 3.1.9). As discussed in Durkin and Diamond (2002), mechanistic assays are generally used to assess the potential for direct action on the endocrine system. DDVP has not been tested for activity as an agonist or antagonist of the major hormone systems (e.g., estrogen, androgen, thyroid hormone), nor have the levels of these circulating hormones been adequately characterized following DDVP exposures. Alterations in the diurnal rhythm of the pituitary/adrenal axis were observed in rats exposed to 2 ppm (approximately 0.3 mg/kg) DDVP in drinking water. Although effects on plasma ChE activity were not noted, levels of plasma adrenocorticotrophic hormones and adrenal cholesterol ester were altered (Civen et al. 1980). In the absence of mechanistic studies of the endocrine system, any judgments concerning the potential effect of DDVP on endocrine function must be based largely on inferences from standard toxicity studies, none of which provide evidence for an endocrine effect.

3.1.9. Reproductive and Teratogenic Effects

No data are available in humans concerning the potential for DDVP-induced reproductive or developmental toxicity. As a small, lipid-soluble molecule, DDVP would be expected to cross the placental barrier and be excreted into breast milk (Desi et al. 1998). According to some studies, exposure to DDVP caused reproductive and teratogenic effects in laboratory animals; on the other hand, there are several breeding studies in which no adverse reproductive or teratogenic effects were observed in rabbits or swine after exposure to DDVP (ATSDR 1997). In a study in which female rats were given intraperitoneal injections of 15 mg/kg DDVP on day 11 of gestation, herniation of the umbilical cord was observed in 3 of 41 offspring from the treated group (Kimbrough and Gaines 1969). The effect was not observed in offspring from the control group (0/65) but the effect is not statistically significant using the Fisher Exact test (p=0.074) – i.e., the conventional criterion for statistical significance is a p-value of ≤ 0.05. In a three-generation study of Wistar rats, oral gavage doses of approximately 1, 1.3, or 1.9 mg/kg-day 5 days/week for 28 weeks found no consistent toxicity (systemic, reproductive, or immunologic) across generations (e.g., birth body weight was statistically decreased in generation 2 and increased in generation 3) (Institäoris et al. 1995, 1997).

When rabbits were treated with 6 mg/kg DDVP during the last 10 days of gestation and the brain tissue of the offspring was examined by electron microscopy, there was an incidence of

immaturity or delay in brain development that was not apparent in the offspring of the untreated rabbits (Dambska et al. 1979). The method of dosing the animals is not specified in this study. Groups of New Zealand White rabbits (16/dose) received DDVP (97% purity in distilled water) orally at dose levels of 0, 0.1, 2.5, or 7.0 mg/kg/day on gestation days 7 through 19 (U.S. EPA 2000a, p. 19). The NOAEL for maternal toxicity was 0.1 mg/kg/day and the LOAEL was 2.5 mg/kg/day, based on decreases in maternal body weight gain during gestation days 7–19. The U.S. EPA (2000a) considered the decrease in weight gain to be biologically significant even though the effect was not statistically significant. A dose-related increase in maternal mortality also was noted at 2.5 and 7 mg/kg/day. Cholinergic signs were observed at 7 mg/kg/day. No adverse developmental effects were noted in the fetuses. Cholinesterase activity was not determined.

An early study by Schwetz et al. (1979) in New Zealand White rabbits and CF-1 mice using the MTD dose (based on signs of cholinesterase inhibition) for both oral (gavage of 5 mg/kg-day DDVP in corn oil on gestation days 5–18 and 60 mg/kg-day DDVP in corn oil on gestation days 5–16 for rabbits and mice, respectively) and inhalation (whole body exposure to atmospheres containing 4 µg/dL (0.4 mg/L or 400 mg/m^3) DDVP for 7 hours/day on gestation days 5–18 or 5–16 for rabbits and mice, respectively) routes of exposure found no teratogenic effects that could be attributed to DDVP. These studies suggest that DDVP is not a selective developmental toxin, since adverse developmental effects only occur at doses that are maternally toxic.

At toxic doses (i.e., where signs of organophosphorus poisoning are evident), DDVP may produce reversible adverse effects on spermatogenesis (WHO 1989). Adverse testicular effects were observed in mice after chronic exposure to average daily doses of 0, 58, or 94.8 mg/kg/day DDVP in drinking water (MRID 41041801 as cited by U.S. EPA 1994). There was a dose-related decrease in the absolute and relative weight of the testes, and testicular atrophy was increased at 94.8 mg/kg/day. In addition, sperm abnormalities were seen in C57BL/C3H mice injected intraperitoneally with 10 mg/kg/day for 5 days (Wyrobek and Bruce 1975). About 6% of the sperm from DDVP-treated animals was abnormal compared to 1.8% of sperm from untreated animals. In a reproductive toxicity study involving male CF-1 mice, groups of 16 mice were exposed to atmospheres containing 0, 30, or 55 mg/m³ (0, 3.3, or 6.1 ppm, respectively) for 16 hours or to 0, 2.1, or 5.8 mg/m³ 23 hours/day for 4 weeks (Dean and Thorpe 1972). No differences between control and treated mice were observed in the number of early fetal deaths, late fetal deaths, or live fetuses found in the pregnant females. The percentage of pregnancies for females mated to males exposed to DDVP was also similar to the controls (73–88%, mean 80.9%). Under these exposure conditions, DDVP does not appear to affect the fertility of male CF-1 mice. No gross or histological evidence of treatment-related damage to reproductive tissues (prostate, testes, epididymis, ovaries, or uterus) was seen in F344 rats (4 or 8 mg/kg/day) or B6C3F1 mice (10, 20, or 40 mg/kg/day) orally exposed to DDVP by gavage for 2 years (NTP 1989).

3.1.10. Carcinogenicity and Mutagenicity

Adequate data regarding the carcinogenic potential of DDVP in humans by any route of exposure are not available. Studies of human populations exposed to DDVP (including workplace and residential exposures) are constrained by the lack of adequate exposure data and other limiting issues. As reported in a series of case studies, some evidence suggests an association between childhood cancer and exposures to DDVP in resin strips during childhood or during gestation (Reeves et al. 1981, Davis et al. 1992, 1993, Liess and Savitz 1995). These studies have been reviewed by U.S. EPA (2000a) which concluded:

> "*[r]eviews of these studies have identified biases and confounders that could explain the observed associations. The Agency concludes that the biases are a more likely explanation for the findings of increased cancer than exposure to resin strips. Additional studies that correct for the control of potential biases and problems of exposure determination are needed before an association between Dichlorvos and childhood cancer can be established*" (U.S. EPA, 2000a, p. 26).

The carcinogenic potential of DDVP has been evaluated in several animal species (mice, rats, dogs, and swine) via the oral route and in rats via the inhalation route. The weight of evidence suggests that the cancer bioassays do not offer sufficient evidence to treat DDVP as a potential human carcinogen (U.S. EPA 2000a,b). DDVP produced positive results in mammalian bioassays for carcinogenicity by the oral, but not the inhalation route of exposure. A cancer bioassay was conducted in which male and female mice were given gavage doses of DDVP (NCI 1977). The doses levels were 10 and 20 mg/kg for males and 20 and 40 mg/kg for females. There was a significant dose-related increase in squamous-cell papillomas of the forestomach in both sexes. In females at the high-dose level, the incidence of squamous-cell carcinomas was significantly greater than in the control group ($p=0.004$ using the Fisher Exact test). In the same study, male rats were given 4 mg/kg/day DDVP by gavage and female rats were given 8 mg/kg/day. A significant ($p<0.001$) dose-related increase in the incidence of acinar-cell adenomas of the pancreas was observed in the males. The increased incidence of fibroadenomas and adenomas of the mammary gland was significant ($p=0.028$) in the females. The increased incidence of the pancreatic acinar cell carcinomas in male rats and squamous cell tumors in male mice reported by NCI (1977) has been discounted by WHO (1989) and Mennear (1994, 1998). The relevance of the sex-specific increase in mononuclear cell carcinoma (MCL) reported by NCI (1977) has also been questioned (Manley et al. 1997, Mennear 1998, U.S. EPA 2000b). The issues of concern regarding the increased incidence of MCL in male rats are not dose-related increases in mortality or disease severity (Mennear 1998), incidence rates among DDVP-treated rats statistically increased as compared to matched controls but within historical control incidence, and similarity in histopathology between the MCL tumors and spontaneous tumors in control animals (Manley et al. 1997). U.S. EPA (2000b) found compelling evidence to disregard the MCL finding in Fisher rats, concluding that "*the high background and variability in the incidence of this tumor, as well as its species and strain specificity, make it an invalid response for human risk assessment*". Two other bioassays conducted on the carcinogenicity of DDVP

after oral exposure are reviewed by IARC (1991). Neither study indicated significant evidence of carcinogenicity (IARC 1991).

DDVP has been tested extensively for mutagenicity, and the results of the tests are available in several reviews (IARC 1979, 1991, Ramel et al. 1980, Mennear 1998, U.S. EPA 2000a,b). Mutagenic effects as well as covalent binding to RNA and DNA have been demonstrated in bacterial systems. Generally, mutagenicity is decreased by the presence of liver microsomal preparations; however, chromosome abnormalities in peripheral lymphocytes have been reported in pesticide workers who use DDVP (no quantitative exposure data are available and this appears to be from workers using a spray formulation of DDVP) (Desi et al. 1998). EPA (2000b) concluded that "*the results from whole animal bioassays supercede the results in vitro tests... [C]ompounds that are positive in mutation tests but do not cause cancer in whole animals should be regulated as noncarcinogens*".

A more detailed review of the cancer and mutagenicity literature database on DDVP is beyond the scope of this risk assessment. Owing to the extraordinary level of effort and Special Agency Reviews of the issue (U.S. EPA 2000a,b), this risk assessment will defer to the EPA's latest position (U.S. EPA 2000a) concerning the carcinogenic and mutagenic potential of DDVP. In that assessment (U.S. EPA 2000a), which included an open meeting to discuss the issues (U.S. EPA 2000b), it was decided that "[*t]he carcinogenicity potential of Dichlorvos has been classified as 'suggestive' under the 1999 Draft Agency Cancer Guidelines and no quantitative assessment of cancer risk is required"*. Thus, this risk assessment for DDVP does not include a quantitative assessment of cancer risk.

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

The available human data, supported by studies on experimental animals, suggest that exposure to DDVP may cause skin irritation or allergic reactions. Human data regarding the dermal effects of DDVP are relatively sparse. In a case report, relatively severe contact dermatitis developed in an adult male after a 1% solution of DDVP leaked onto his skin (Bisby and Simpson 1975). This effect was accompanied by signs of cholinergic toxicity, including fatigue, dizziness, and labored respiration. Cases of dermatitis and skin sensitization due to DDVP have been described in workers handling and spraying different types of pesticides and cross-sensitization with certain pesticides has been seen (WHO 1989).

The data from animal testing supports the results of human case reports. In New Zealand white rabbits, the application of an aqueous solution of 5–20% DDVP to the skin caused relatively severe irritation (Arimatsu et al. 1977). In a skin sensitization assay, 1% DDVP in olive oil induced no visible effects in male albino guinea pigs (Kodama 1968). In a guinea pig assay for allergenicity, 35% of the tested guinea pigs had a positive response to a 0.5% solution of DDVP (Fujita 1985). In a sensitization assay, Ueda et al. (1994) reported that 1% DDVP was a threshold irritation concentration in guinea pigs and that cross-sensitization occurred between DDVP and triforine. WHO (1989) reported that in Hartley guinea pigs the primary irritant threshold limit value for DDVP was ≥2%.

3.1.12. Systemic Toxic Effects from Dermal Exposure

Most of the systemic effects observed after dermal exposure of laboratory animals (including monkeys, rats, and chickens) to DDVP were the result of the neurotoxicity of this chemical. In its risk assessment for DDVP, U.S. EPA (2000a) selected studies for short-term and intermediate-term risk assessment that reflect the systemic toxicity resulting from dermal exposures to DDVP. In both of these studies, the toxicity of DDVP is secondary to inhibition of cholinesterase activity. Data concerning the dermal absorption kinetics of DDVP are discussed in Section 3.1.3.2.

A number of fatalities have been reported from dermal exposures to concentrated formulations of DDVP (spilling or splashing onto skin) (WHO 1989). The data suggests that, in those cases where the spilled solution was immediately washed off, the victims developed symptoms of organophosphorus poisoning but they recovered after treatment (WHO 1989). Such exposures are not relevant to this risk assessment, as the encapsulation of DDVP in PVC used in Vaportape II precludes rapid exposure to high doses of DDVP.

3.1.13. Inhalation Exposure

Exposure of pesticide manufacturing plant workers to concentrations in the air of up to 0.5 mg/m^3 were without clinical effects, and no, or only insignificant, inhibition of blood ChE activity was noted (WHO 1989). When DDVP is used properly, air levels of 0.01–0.03 ppm are achieved (ATSDR 1997). This level kills most insects within 1 hour; whereas, in human volunteers, exposure at about 20 times this level (0.23 ppm) for 2 hours a day for 4 days had no harmful effects (ATSDR 1997). Consistent with the human exposure data, harmful effects have not been seen in laboratory animals exposed to air levels of dichlorvos below 0.5 ppm (about 4.5 mg/m^3) (ATSDR 1997), and exposure of laboratory animals to DDVP air concentrations between 0.2–1 mg/m^3 do not affect ChE activity significantly (WHO 1989). In a 2-year study in rats, breathing air every day containing low-to-moderately high levels (0.006–0.6 ppm or about 0.05 to 5 mg/m^3) of DDVP had no effect on survival or general health (ATSDR 1997). Generally, the systemic effects observed after inhalation exposure of laboratory animals to higher levels of DDVP were the result of the neurotoxicity (cholinesterase inhibition) (U.S. EPA 2000a). Chronic inhalation exposure of laboratory animals to DDVP produced no compound-related pulmonary toxicity (U.S. EPA 2000a).

EPA (1994) selected the chronic inhalation study in rats (Blair et al. 1976) as the basis for establishing an RfC for DDVP. Groups of 50/sex/group Carworth E Farm (CFE) rats were exposed (whole body exposures) for 23 hours/day, 7 days/week to DDVP vapor (>97% purity) at atmospheric concentrations of 0, 0.05, 0.5, and 5 mg/m^3 for 2 years. The rats were observed for clinical signs of toxicity, hematology, and clinical chemistry. Plasma, RBC, and brain cholinesterase activity were determined at study termination, but not prior to the study. No clinical signs of toxicity were observed, and no organ weight or organ to body weight changes or hematological changes were associated with DDVP exposure. Body weights were decreased as compared to control rats in high-dose male (up to 20% vs. control) and female rats (up to 14% vs. control) for large portions of the study. Dose-dependent reductions in plasma, RBC, and

brain cholinesterase activity were observed. This study establishes a NOAEL of 0.05 mg/m³ and a LOAEL of 0.5 mg/m³ based on reductions in brain cholinesterase activity (U.S. EPA 2000a).

3.1.14. Inerts and Adjuvants

As discussed in Section 2.2, the DDVP used in gypsy moth control programs is contained in a multi-layered polyvinyl chloride (PVC) strip. The manufacturer (Hercon 2004) indicates that the product contains 10% DDVP, 0.75 % related compounds (Section 3.1.15), and 89.25% inert ingredients. The only toxicity data available on this strip itself (i.e., without DDVP) is an acute oral toxicity study in rats (Braun and Killeen 1975). This study used a DDVP-free strip ground to a "grayish-green powder". The strip was tested at the limit dose of 5,000 mg/kg bw by gavage with a 14-day post-dosing observation period in 5 male and 5 female rats. No adverse effects were noted in any of the rats based on mortality, gross observations, body weight gain, and gross necropsy. While this single study has its limitations, it suggests that the PVC strip alone (i.e., without DDVP) is unlikely to produce acute adverse effects. Given the limited nature of the exposure scenarios assessed herein, these data may be sufficient information for the likely exposure scenario (i.e., a child putting a strip in his/her mouth). Section 3.1.17 focuses on the toxicity studies concerning DDVP embedded in the PVC strips.

3.1.15. Impurities and Metabolites

The product label Hercon (2004) specifies that, in addition to DDVP (10%), each strip contains 0.75% compounds that are related to DDVP. Further details are not provided on the label; nonetheless, the impurities in commercial DDVP have been characterized (Gillett et al. 1972a; IARC 1991). The impurities include: Dipterex (O,O-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate); O,O-dimethyl 2-chlorovinyl phosphate; O,O-dimethyl methylphosphonate; O,O,O-trimethyl phosphate; and trichloroacetaldehyde. These impurities are known to be or are likely to be toxic (Gillett et al. 1972a, WHO 1989). These impurities are encompassed in the risk assessment because the effect levels are based on studies that used commercial grade DDVP. Consequently, the results of these studies are directly applicable to the risk assessment for human health.

3.1.16. Toxicologic Interactions

The major toxicologic interaction of concern is concurrent exposure to other cholinesterase inhibitors (e.g., organophosphate or carbamate insecticides) or cholinomimetic agents (e.g., agents such as pilocarpine or carbachol that mimic the action of acetylcholine). In either case, simultaneous exposure would likely enhance the cholinergic toxicity produced by DDVP. Potentiation studies using DDVP in combination with 22 other organophosphate pesticides, however, found little or no potentiation (WHO 1989). Chemicals that react with the serine residue at the active site of the "A"-type esterases (e.g., diisopropylfluorophosphate [DEP]) could also increase the toxicity of DDVP by interfering with its metabolism (ATSDR 1997).

In addition, experimental data suggest that repeated exposures of rats to DDVP (5 mg/kg/day by intraperitoneal injection for 30 consecutive days) depletes brain glutathione levels (Julka et al. 1992). Reduced glutathione levels may decrease the rate of detoxification of DDVP by the

glutathione-dependent metabolic pathways. The toxicologic significance of depleted brain glutathione on DDVP metabolism is not known. In contrast with the potentiation of DDVP toxicity observed when rats are pretreated with diethylmaleate (Fukami 1980), Costa and Murphy (1984) reported that pretreatment with 600 mg/kg acetaminophen (which is also detoxified by and thus reduces glutathione levels) did not have any effect on the toxicity of DDVP. Although no data are available, these experiments suggest that repeat exposure to DDVP (resulting in a depletion of glutathione levels) may increase an organism's susceptibility to toxicity by another chemical if that chemical is also detoxified by glutathione-dependent pathways.

3.1.17. Studies on PVC Formulations of DDVP

In the EPA risk assessment for DDVP (U.S. EPA 2000a), EPA noted that DDVP resin strips (such as the Vaportape II strip used in USDA programs) "*account for a very small proportion of total incidences* [e.g., reports of poisonings]*, about 33 cases per year (1% of total incidences). Incidence reports involving exposure to resin strips usually do not involve any significant acute symptoms that would require medical treatment*". In a review of DDVP-impregnated PVC strips (Gillett 1972a,b concluded that "*even when chewed or applied directly to the skin for short intervals, the strips do not release excessive or hazardous amounts of DDVP*".

When DDVP was administered orally to human volunteers (single or repeated doses of a slow-release PVC formulation), significant inhibition of RBC ChE activity was found only at 4 mg/kg body weight or more (Hine and Slomka 1970; Slomka and Hine 1981). Single oral doses (1–32 mg/kg) of DDVP in a slow-release PVC formulation was administered to 107 male volunteers. Measurable reductions in erythrocyte ChE activity was observed at dose levels above 4 mg/kg, with a maximum reduction of 46% at 32 mg/kg. Plasma ChE activity was affected at lower doses, with 50% reduction at 1 mg/kg and about 80% reduction at 6 mg/kg or more. Repeated oral doses of 1–16 mg/kg bw per day were given to 38 male volunteers for up to 3 weeks. Plasma ChE activity was depressed at all dose levels, and RBC AChE activity depression was dose-related and statistically significant at doses of 2 mg/kg or more. Blood cell count, urine, liver function, prothrombin time, and blood urea nitrogen were all normal (Hine and Slomka 1968, 1970, Slomka and Hine 1981, WHO 1989). Among these individuals, the clinical signs of DDVP exposure were minimal (nausea, diarrhea, lassitude, restlessness, and light-headedness).

Data from 32 rhesus monkeys receiving orally administered DDVP in PVC resin (as an anthelminthic) at 0, 5, 10, 20, 40, or 80 mg/kg once daily or 0, 8, or 20 mg/kg twice daily for 10 to 21 days support the human data (Hass et al. 1971). None of the monkeys died or exhibited debilitating symptoms of organophosphorus poisoning, although some cholinergic effects were noted (a loss of appetite and emesis [LOAEL = 20 mg/kg]; diarrhea and salivation [LOAEL = 80 mg/kg]). A semi-quantitative assay for cholinesterase activity demonstrated inhibition. Studies in swine treated with DDVP-impregnated pellets (veterinary use as anthelminthic) suggest that DDVP is absorbed from the pellets after oral exposure (Jacobs 1968, Potter et al. 1973). Neither study was reported in sufficient detail to develop dose-response relationships.

Two reproduction studies investigated exposure to PVC-DDVP formulations. In one of the studies, swine were exposed to 5 or 25 mg/kg/day DDVP during the last 30 days of gestation (Stanton et al. 1979). Sows and fetuses were monitored for changes in ChE. Both plasma ChE and RBC AChE were inhibited in sows, and brain AChE was increased in fetuses. In a separate experiment conducted by these investigators, there were no significant effects on reproductive capacity in sows treated with 25 mg/kg/day DDVP during the last 30 days of gestation. In an abstract concerning DDVP encapsulated in PVC, Vogin (1971) reported that no adverse effects on reproduction or developmental parameters were observed in dams exposed to DDVP concentrations that did not cause maternal toxicity (up to 12 mg/kg). Maternal toxicity was evident in dams treated with 34 mg/kg. This abstract also employed exposures to PVC resin and dioctylphthalate to assess the potential developmental toxicity of inerts. No teratogenic effect was reported for any exposure regimen.

When DDVP pesticide strips were used in hospital wards, exposure of hospitalized adults and children, as well as healthy pregnant women and newborn babies, did not produce any significant effects on plasma ChE or RBC AChE activity. Exposures were estimated TWA concentrations of 0.05, 0.152, and 0.159 mg/m^3 based on 18 hours/day (Vigliani 1971). Only those subjects exposed 24 hours/day to concentrations above 0.1 mg/m^3 or patients with liver insufficiency showed a moderate decrease in plasma ChE activity (Cavagna et al. 1969). Cavagna et al. (1969) also calculated DDVP inhalation exposure doses (based on inhalation volumes of 10 m^3/day for adults and 1.4 m^3/day for children and continuous exposures) that would be required to produce a significant reduction in plasma ChE activity (25–54% reduction in activity) for healthy adults and children (approximately 0.03 mg/kg-day) and adults and children with liver insufficiency (approximately 0.006 mg/kg-day). Note that these exposure doses are not anticipated to produce signs or symptoms of cholinesterase inhibition (Cavagna et al. 1969). No significant effects on plasma ChE or RBC AChE activity were observed in people exposed to the recommended rate of one strip per 30 m^3 in their homes over a period of 6 months, even when the strips were replaced at shorter intervals than that normally recommended (Zavon and Kindel 1966). The maximum average concentration in the air of the homes was approximately 0.1 mg/m^3 (WHO 1989). In factory workers exposed to an average of 0.7 mg/m^3 for 8 months, significant inhibition of plasma ChE and RBC AChE activity was found (WHO 1989).

In a study evaluating the effects of 30 minutes of dermal exposure to a DDVP pest strip on AChE activity, no dermal effects were noted in 21 individuals (Zavon and Kindel 1966). Zavon and Kindel (1966) also reported no inhibition of plasma or erythrocyte cholinesterase from the 30 minute dermal exposure as well as 5 consecutive days of 30 minutes of continuous dermal exposure to DDVP resin strips. EPA (1981) provides a summary or exposure incidents involving DDVP in the general public. The reports involving DDVP-impregnated resin strips involved dermal contact which largely resulted in DDVP-induced allergic reactions or contact dermatitis (this is consistent with the effects of DDVP reported in dermal contact bioassays as described in Section 3.1.12). Flea collar dermatitis (primary contact dermatitis) has been reported in dogs and cats wearing DDVP-impregnated PVC flea collars (Muller 1970), and four people who handled dogs wearing flea collars containing 9–10% DDVP developed contact dermatitis (patch tests

using 0.25–1% DDVP in these individuals were positive). The data suggest that a very small proportion of the general population is susceptible to dermal irritation by DDVP (WHO 1989).

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview.

Under normal conditions, exposure to both workers and members of the general public should be negligible. Workers will handle strips only during the assembly of milk carton traps. If workers wear gloves and assemble the traps outdoors or in very well ventilated rooms, both inhalation and dermal exposures should be negligible. Inhalation exposure to DDVP during transport of the traps should also be negligible if the traps are not transported inside of the passenger compartments of vehicles. Worker exposures will also be limited in most programs because foil wrapping in which the strip is distributed will not be removed until after the trap is transported to the field. Milk carton traps will generally be placed about four feet above the ground and exposure of members of the general public to DDVP contained in the milk carton traps should also be negligible except in the case of intensional tampering.

Notwithstanding the above assertions, exposure assessments are developed for workers who do not use gloves in the assembly of the milk carton traps and who assemble the traps indoors, remove the protective foil strip during assembly, and transport the traps in the passenger compartments of vehicles. All of these exposure scenarios should be considered atypical and some are extreme. The intent is to illustrate the consequences of mishandling or imprudent handling. During assembly, the central estimate of dermal exposures in workers not wearing gloves leads to an absorbed dose of about 0.0008 mg/kg with a range of about 0.0003 mg/kg to 0.004 mg/kg. Inhalation exposures in workers may be highly variable depending on the ventilation rates in an enclosed space and the number of traps that are handled. Based on the handling and transport of 75 traps, inhalation exposures could reach up to about 0.6 mg/m^3 in an enclosed and unventilated room and up to about 1.8 mg/m^3 in the passenger compartment of a vehicle. These exposure assessments are based on several site and situation specific assumptions which are intended to reflect plausible upper bounds of exposures.

Exposure assessments are also developed for children who might come in contact with an accidentally discarded or misplaced strip. Estimated dermal doses are much higher than those for workers: a central estimate of about 0.02 mg/kg with a range of 0.003 mg/kg to 0.1 mg/kg. Oral exposures from a small child sucking on the pest strip are about a factor of 10 higher than dermal exposures: a central value of about 0.2 mg/kg with a range of 0.04 mg/kg to 0.6 mg/kg.

Under normal circumstances, the use of DDVP in PVC strips is not likely to result in contamination of water or other materials that might be consumed by members of the general public. Nonetheless, an exposure assessment is developed for the accidental contamination of a small pond by a pest strip. In this scenario, dose estimates range from about 0.000003 mg/kg to 0.00007 mg/kg with a central estimate of about 0.00001 mg/kg.

3.2.2. Workers

3.2.2.1. General Considerations – The EPA (2000a) concluded that human exposures would be negligible from DDVP-impregnated strips in insect traps (such as those used in USDA programs). Consequently, the EPA (2000a) did not quantitatively assess the exposure or

potential risks posed by the use of PVC formulations of DDVP for any route of exposure. While this may be a reasonable approach, the current risk assessment develops quantitative exposure assessments for both workers and the general public that could occur in cases of poor handling practices.

The milk carton traps can be assembled in two stages. The most time consuming stage is the carton assembly, in which two pre-cut perforated pieces of heavy waxed paper, similar to those used in milk cartons, are configured. In the second stage, the DDVP strip and disparlure wick are attached to the twist tie, and the twist tie is placed in the trap. The second stage should proceed much more rapidly than the first. During assembly, two routes of exposure may be significant, inhalation and dermal. As discussed in the program description (Section 2.2), however, both routes of exposure will be negligible if proper handling procedures are followed (that is, if the strips are installed outdoors or in a well ventilated area, if foil wrapping in which the strip is distributed is removed until after the trap is transported, and dermal contact with the strip is avoided).

3.2.2.2. Inhalation Exposures – During normal use and assembly, either outdoors or in well ventilated areas, inhalation exposures to DDVP should be negligible. The material safety data sheet for VaporTape II (Hercon 1993) calls for local exhaust and respirators under conditions of continuous handling. Estimates of concentrations of DDVP in air from release of DDVP by VaporTape strips under different conditions of ventilation can be based on estimates of release rates (Hercon 1994) and a more general air model for DDVP pest strips proposed by Gillett et al. (1972a).

Hercon (1994) conducted a study on the release of DDVP from Vaportape II strips. In this study, two samples (referred to as **A** and **B**) were weighed and assayed for DDVP at various intervals for up to 12 weeks after placement outdoors. The results, expressed as the proportion of DDVP remaining in the strip at various intervals, are detailed in Worksheet A01. As also detailed in Worksheet A01, the release data fit a first order model extremely well with an adjusted squared correlation coefficient of 0.97 and a p-value of 2×10^{-23}. The estimated first-order release coefficient is 0.04 day^{-1} with very narrow confidence intervals – i.e., 0.037 to 0.043 day^{-1}.

Gillett et al. (1972a) proposed the following model for estimating concentrations of DDVP in air from the release of DDVP from pest strips:

$$C_t = \frac{8}{\pi^2}\,\frac{M_0}{Va(1+\gamma)}\,\frac{\exp(-\lambda t) - \exp\left(\dfrac{-\left(kRH + \dfrac{At}{Va}\right)}{1+\gamma}\,t\right)}{\dfrac{\left(kRH + \dfrac{At}{Va}\right)}{\lambda\,(1+\gamma) - 1}} \qquad \text{(Eq. 3-1)}$$

The terms in the above equation are defined as follows:

$\boldsymbol{t}$	time after start of release
C_t	concentration of DDVP in air at time, t (days)
M_0	mass of DDVP in strip or strips at time zero (mg)
Va	volume of room or other space (m^3)
$\boldsymbol{\gamma}$	apparent adsorption coefficient of DDVP on to surfaces
exp($\boldsymbol{x}$)	the exponential function, $\boldsymbol{e}^{\boldsymbol{x}}$, where $\boldsymbol{e}$ is the constant 2.718 and $\boldsymbol{x}$ is any numeric expression
$\boldsymbol{\lambda}$	first-order release rate constant ($days^{-1}$)
$\boldsymbol{RH}$	relative humidity (proportion)
A_t	air flow rate (m^3/day)
$\boldsymbol{k}$	first-order vapor phase hydrolysis rate ($days^{-1}$)

The parameters used in the model are summarized in Table 3-2. The fit of the Gillett et al. (1972a) model to the data from Slomka (1970) using the apparent adsorption coefficient ($\boldsymbol{\gamma}$) of 37.5 is illustrated in Figure 3-1 (which is in turn taken from Worksheet A02b). Technical details of the application of the model and optimization of the model parameter for adsorption ($\boldsymbol{\gamma}$) are given in Appendix 1.

For the current risk assessment, two scenarios are considered for inhalation exposures of workers to DDVP: assembly of traps with strips in a garage and driving in a vehicle containing assembled traps with the strips. Both scenarios assume that the worker has removed the protective foil from the strip during assembly of the trap. These exposure scenarios are detailed in Worksheets A03a (garage) and A03b (vehicle). It should be noted that these exposure assessments are based on a number of plausible but conservative exposure assumptions – i.e., number of traps assembled or transported, volume of the space in which the traps are assembled or transported, and the ventilation rates of these spaces. The worksheets in which these exposure assessments are given are designed so that these parameters may be varied and applied to specific uses of the DDVP strips in specific USDA programs.

A major factor in exposure will be the number of traps that are assembled. In the previous risk assessment (USDA 1995a), it was assumed that a workers would assemble up to 75 traps at a time. No more recent information has been encountered on the number of traps that might be assembled by a worker or workers and the value of 75 traps is maintained in the current risk assessment.

For exposures in a garage involving the assembly of the milk carton traps, the dimensions of the garage are assumed to be 1,500 ft^3 (10 feet · 10 feet · 15 feet) or 42.48 m^3 [1 ft^3=0.02832 m^3]. For the exposure assessment involving transport of the strips in a vehicle, the volume of the

driving cabin is assumed to be 160 ft^3 (8 feet · 5 feet · 4 feet) or 4.5 m^3. Again, these assumptions are somewhat arbitrary but are identical to the assumption used in the previous risk assessment (USDA 1995a).

The other major assumptions used in these exposure scenarios involve ventilation rates and release rates. The release rate is taken as 0.04 day^{-1} from the study by Hercon (1994) discussed above and detailed in Worksheet A01. It should be noted that the study by Hercon (1994) was conducted outdoors over a period of 12 weeks. Hercon (1994) does not specify the average temperature or range of temperatures. As discussed in Gillett et al. (1972a), the release rate of DDVP from PVC test strips will increase with increasing temperature, doubling from a temperature of 25°C to 38°C. This variability is not explicitly incorporated into the model used in this risk assessment (Eq. 3-1) and release rates higher than 0.04 day^{-1} are possible at high ambient temperatures.

Ventilation rates are likely to be highly variable. In most cases, it is likely that the milk carton traps will be assembled outdoors and will be transported in a cargo area and not in the driving cabin. In such cases, inhalation exposure would likely be negligible. For the purpose of illustrating the consequence of assembling traps in a garage or similar structure or transporting assembled traps in a vehicle, three ventilation rates (number of air turnovers per day) are used for each scenario. Rates of 0 day^{-1} (no ventilation) and 60 day^{-1} (poor ventilation) are used in both scenarios. An additional rate of 300 day^{-1} is used in the garage scenario and an additional rate of 3000 $day^{-1}$is used in the vehicle scenario. These rates are referred to as *"Adequate"* in Worksheets A03a and A03b. As discussed further in Section 3.4.2, this term is used because these ventilation rates lead to concentrations in air that are about 0.1 mg/m^3, the chronic NOAEL from animal studies and the TLV recommended by ACGIH (2004).

As detailed in Worksheet A03a, the garage scenario models concentrations over a 24 hour period. This duration period is selected under the assumption that traps might be stored for a day prior to use. The modeled concentrations reach up to about 0.5 mg/m^3 for no ventilation and 0.3 mg/m^3 for poor ventilation. As noted above, peak concentrations of 0.1 mg/m^3 are obtained with a ventilation rate of about 300 day^{-1}. The vehicle scenario (Worksheet A03b) covers a period of only 6 hours. It is likely that the duration of transport would typically be much less. Peak concentrations are somewhat higher – 1.8 mg/m^3 for no ventilation and about 1.5 mg/m^3 for poor ventilation. It is unclear if the no ventilation or poor ventilation assumptions are reasonable for a vehicle. As discussed by Fedoruk and Kerger (2003), concentrations of volatile organic compounds in vehicles suggest that substantial air turnover rates are likely in vehicles even when the ventilation system is turned off and the windows are closed. Quantitative estimates of air turnover rates in vehicle passenger cabins, however, have not been encountered. Nonetheless, it seems that turnover rates of 0 day^{-1} or 60 day^{-1} will lead to overestimates of concentrations of DDVP in the air of passenger compartments. Adequate ventilation for a vehicle is defined as a turnover rate of 3000 day^{-1}, the rate required to reach a concentration in air of about 0.1 mg/m^3.

3.2.2.3. Dermal Exposures – For assessing the likelihood of systemic toxic effects from dermal exposures, such as handling a pest strip during assembly, some estimate of absorbed dose is necessary. The method for making such an assessment for DDVP test strips, however, is highly uncertain.

As an individual manipulates the strip, some material will be transferred to the surface of the skin. Some of the chemical will be absorbed and some will volatilize. Assuming that the nature of the manipulation is such that a film of DDVP is maintained on the contaminated surface, Fick's first law may be used to estimate absorption (U.S. EPA/ORD 1992). Fick's first law requires an estimation of the K_p in cm per hour, the concentration of the chemical in a solution in contact with the skin, the area of the body surface that is contaminated, and the duration of exposure. There is no experimentally determined K_p for DDVP. Based on structure-activity relationships proposed by the U.S. EPA/ORD (1992), K_p for DDVP is may be estimated at about 0.00090 cm/hour with a 95% confidence interval of 0.00061 cm/hr to 0.0013. Details of these calculations are given in Appendix 2.

In this and other similar scenarios considered in this risk assessment, the DDVP is not in solution; instead, the skin is in contact with neat or undiluted DDVP. Following the recommendations of U.S. EPA/ORD (1992), the functional concentration of DDVP on the surface of the skin is assumed to be the solubility of DDVP in water, 10 mg/mL (Table 2-2) – i.e., the concentration of DDVP in pore water of the skin will be limited by the solubility of the chemical in water.

For workers wearing gloves, dermal absorption will be negligible. For workers who do not wear gloves, it is possible that the tips of the fingers and perhaps other surfaces on the hands would be contaminated. The most likely surface for contamination would be the finger tips. The precise area that might be contaminated, however, is difficult to estimate. The finger tip of each digit will be taken as 1 cm^2, except for the thumb that will be taken as 2 cm^2. Thus, the total surface area of the finger tips of both hands will be taken as 12 cm^2. This value will be used to calculate both lower and central estimates of absorbed dose. To account for the potential contamination of other parts of the hand, the upper range of exposed surface area will be taken as 24 cm^2. The duration of exposure is difficult to estimate. Most of the time spent in assembling the milk carton trap will not involve the DDVP strip. For this exposure assessment, a central estimate of 0.5 hours of total contact time with the strip is used and the range is taken as 0.25 hours to 1 hour. As detailed in Worksheet B01a, the assumptions used in this exposure scenario lead to estimates of absorbed dose of about 0.0008 mg/kg with a range of about 0.0003 mg/kg to 0.004 mg/kg.

3.2.3. General Public

3.2.3.1. General Considerations – Milk carton traps contain the strip of Vaportape II attached to a twist tie or simply placed in the bottom of the trap. The DDVP strip can be accessed easily and removed. As summarized by U.S. EPA (2000a, p. 26), incidents involving contact with DDVP resin strips have been reported but these incidents account for only a small proportion of the total

incidents involving DDVP (1% or about 33 cases per year) and the reported incidents involving DDVP strips typically to do not lead to overt signs of toxicity that require medical treatment.

In the current risk assessment, two routes of exposure are considered for the general public: dermal contact and ingestion. Milk carton traps will generally be placed about four feet above the ground (Leonard 2004) and exposure of members of the general public to DDVP contained in the milk carton traps should also be negligible except in the case of intensional tampering. Although any member of the general public could tamper with a trap, incidents such as these seem to be more plausible for children, compared with adults. While the traps may be place out of the reach of young children, the potential for exposure to the DDVP strip could occur if traps were accidentally dislodged or misplaced. In addition, using children as the exposed group is conservative because dose estimates for children, in units of mg/kg body weight, will be higher than those for adults.

3.2.3.2. Dermal Contact – The exposure assessment for dermal contact with a VaporTape II strip is detailed in Worksheet B01b. This scenario is very similar to that for dermal contact in a worker (Worksheet B01a). The major differences involve body weight, the dermal surface area that is considered, and the duration of exposure. The body weight is taken as 13.3 kg, the standard value for a 2-3 year old child (U.S. EPA/ORD 1996). In this scenario, it is assumed that a young child comes in contact with a pest strip and holds the strip against the surface of the skin for a period of time. Thus, the exposed skin surface area is taken as the dimensions of the strip – i.e., 1" x 4" inches or about 26 cm^2). The duration of exposure must be set somewhat arbitrarily. It does not seem reasonable to assume that a 2-3 year old child would be unsupervised for a prolonged period of time. Consistent with the approach taken in the 1995 risk assessments (USDA 1995a), the central estimate of exposure will be taken as 1 hour with an upper range of 4 hours. In the current risk assessment, a lower range of 15 minutes (0.25 hours) is also used and may be a more reasonable estimate of a plausible duration of exposure. Other assumptions and calculations are identical to those in the corresponding worker exposure assessment (Worksheet B01a, Section 3.2.2.3). As indicated in Worksheet B01b, this exposure assessment for a young child handling a DDVP strip leads to an estimated dose of about 0.02 mg/kg with a range of 0.003 mg/kg to 0.1 mg/kg.

3.2.3.3. Oral Exposure to DDVP Strip – As with dermal exposure, it is unlikely that children would experience any oral exposure to DDVP strips. The strips are placed within the milk carton traps and 2-3 year old children will generally be closely supervised. Thus, this exposure assessment for oral exposure, as with the above scenario for dermal exposure, should be regarded as accidental.

An assessment of oral exposure might be based on incidental sucking on a pest strip. The amount of DDVP that a child might absorb will depend on the proportion of the strip that is in the mouth, the release rate of DDVP from the strip, and duration of the activity. The durations will be taken as the same as in the dermal exposure scenario, a central estimate of 1 hour with a range of 0.25 to 4 hours. The initial release rate will be taken as 0.015 $hour^{-1}$. This is calculated

from the study by Slomka and Hine (1981) which indicated that approximately 30% of the DDVP was released in the first 24 hours – i.e., k = -ln(1-f)/t = ln(1-0.3)/24 hours = 0.01486 $hour^{-1}$.]. The proportion of the strip that might be in the mouth of the child will be taken as 0.25 – i.e., a area of about 1 square inch. As indicated in Worksheet B02, this exposure assessment results in estimates of absorbed doses of about 0.2 mg/kg with a range of 0.04 mg/kg to 0.6 mg/kg. This scenario would also involve some dermal exposure. As indicated in Section 3.4, any plausible dermal exposure would likely be much less than the oral exposure and would have no impact on the characterization of risk.

3.2.3.4. Oral Exposure to Contaminated Water – Under normal circumstances, the use of DDVP in PVC strips is not likely to result in contamination of water or other materials that might be consumed by members of the general public. In the recent risk assessment by U.S. EPA (2000a), no exposure assessment for water contamination by DDVP in PVC formulations is presented.

The approach taken by U.S. EPA (2000a) seems reasonable in that the slow release DDVP from the test strip and rapid hydrolysis of DDVP in water is likely to limit the concentration of DDVP in ambient water. For example, the halftimes for the hydrolysis of DDVP in water range from about 11.65 days at pH 5 to 0.88 days at pH 9, with a hydrolysis halftime of 5.19 days at pH 7 (U.S. EPA 1999a, p. 3). These values correspond to hydrolysis rates – i.e., $k = \ln(2)/t_{50}$ – of 0.06 day^{-1} [pH 5], 0.13 day^{-1} [pH 7], and 0.78 day^{-1} [pH 9]. All of these hydrolysis rates are more rapid than the release rate of DDVP in air from the Hercon pest strip – i.e., 0.04 day^{-1} as discussed in Section 3.2.2.2.

For this risk assessment, the assumption will be made that a VaporTape strip accidentally contaminates a small pond (e.g., it is inadvertently dropped into a pond during placement of a trap or a trap is dislodged and falls or is blown into a pond). No data are available to directly estimate the amount of DDVP that might be released over the course of a single day. For this exposure assessment, the assumption will be made that 30% of the DDVP in a fresh strip might be released over the course of a single day. This is based on the study by Slomka and Hine (1981), discussed in Section 3.1.4, in which 30% of the DDVP was released from a pest strip into gastric juices over a 24 hour period. Thus, the central estimate of the amount of DDVP in water is taken as 177 mg [590 mg × 0.3]. The upper range of the amount of DDVP in water is taken simply as the amount of DDVP in a new pest strip – 590 mg. The selection of a lower is somewhat arbitrary and a value of 10% or 59 mg is used. Other details of this exposure assessment are given in Worksheet B03 and involve standard assumptions concerning the size of the pond and the amount of water that might be consumed. These assumptions are standard in risk assessments (SERA 2001). As detailed in Worksheet B02, dose estimates range from about 0.000003 mg/kg to 0.00007 mg/kg with a central estimate of about 0.00001 mg/kg.

As noted above, this very simple exposure scenario does not consider the degradation or dissipation of DDVP. As discussed further in Section 3.4, however, this exposure assessment leads to concentrations in water that are far below a level of concern. Thus, the overestimates of

concentrations in water developed in this section have no impact on the risk characterization for potential effects in humans.

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview

The extensive toxicology data base has been evaluated by a number of governmental organizations including the U.S. EPA, the Agency for Toxic Substances and Disease Registry (ATSDR), National Institute for Occupational Safety and Health (NIOSH), Occupational Safety and Health Administration, and the World Health Organization. Following the approach taken in most USDA risk assessments, these sources are used for selecting levels of acceptable exposure. Because all of the scenarios considered in this risk assessment involve only acute exposures, only acute exposure criteria are considered.

For both oral and dermal exposures, the acute RfD established by the U.S. EPA, 0.0017 mg/kg, is used for the risk characterization. This is based on an acute oral NOAEL of 0.5 mg/kg from a study in rats with the application of an uncertainty factor of 300. Acute exposure criteria proposed by other groups are comparable to but somewhat higher than the acute RfD. Because some of the accidental acute exposures may substantially exceed the acute RfD, some attempt is made to characterize the consequences of high oral exposures. A human NOAEL of 1 mg/kg for AChE inhibition has been identified. While this NOAEL is not used to modify the acute RfD, it can be used to assess plausible consequences of exceeding the RfD. The human data on DDVP, although extensive, are not sufficient to identify a minimal lethal dose. For the current risk assessment, the lowest reported lethal dose (16 mg/kg) is used to assess the plausibility of observing serious adverse effects in cases of accidental over-exposure to DDVP.

A number of inhalation criteria for DDVP are available. Since potentially significant inhalation exposures are likely only in workers, the occupational exposure criterion of 0.1 mg/m^3 proposed by American Conference of Governmental Industrial Hygienists is used. This value is a factor of 10 below the occupational criteria proposed by NIOSH and OSHA.

3.3.2. Acute Exposures

3.3.2.1. Acute Oral – As summarized in Section 3.1.4, the U.S. EPA (2000a) bases the acute oral RfD for DDVP on the study by Bast et al. (1997) in which no effects, including assays for alterations in behavior, were noted at 0.5 mg/kg but neurological effects related to AChE inhibition were noted at 35 mg/kg. In deriving the acute RfD, the U.S. EPA (2000a, p. 18) used an uncertainty factor of 300 and recommended an acute RfD of 0.0017 mg/kg/day [0.5 mg/kg ÷ 300 = 0.0017 mg/kg]. ATSDR (1997) has recommended a somewhat higher acute oral minimal risk level (MRL) – a value that is analogous to the RfD – of 0.004 mg/kg/day. This is based on a 14-day LOAEL of 4 mg/kg/day in which brain AChE was inhibited by 44%. The MRL was calculated using an uncertainty factor of 1000 (ATSDR 1997, pp. 83-84).

As also discussed in Section 3.1.4, the study by Stanton et al. (1979) suggests that DDVP in a PVC formulation will be much less toxic than unformulated DDVP. The extent of the difference in toxicity, however, is difficult to quantify. For unformulated DDVP, the LD_{50} value was 157 (113–227) mg/kg with no mortality observed at 56 mg/kg. For the DDVP-PVC formulation, no deaths occurred at doses of up to 1000 mg/kg, although signs of toxicity consistent with AChE

inhibition were observed at doses of 320 mg/kg and 1000 mg/kg using the DDVP-PVC formulation. No tremors or salivation were observed at doses of 240 or 180 mg/kg of the DDVP-PVC formulation. Stanton et al. (1979) do not provide comparative data the extent of AChE inhibition in unformulated DDVP and the DDVP-PVC formulation.

As detailed in Section 3.2.3.3, estimates of acute oral exposure for a small child sucking on a pest strip are far above the acute RfD of 0.0017 mg/kg. Thus, the potential for more severe effects must be considered. Based on the recent study by Gledhill (1997), no changes in AChE activity and no signs of toxicity were seen in a group of 6 men administered DDVP in a gelatin capsule at an approximate dose of 1 mg/kg. This is a factor of about 600 above the acute oral RfD. This study is unpublished and was submitted to the U.S. EPA by a registrant. In the U.S. EPA (2000a) human health risk assessment, the MRID number for this study is cited but the results of the study are not discussed specifically. For the current risk assessment, a dose of 1 mg/kg from the Gledhill (1997) study is used qualitatively to characterize the risks of exposures that are not likely to produce clinically significant effects.

For many pesticides, exposures that would be associated with severe and possibly fatal effects often can be estimated from poisoning reports. Most reports of fatal exposures to DDVP, however, do not provide sufficient information to estimate a lethal dose in humans. An approximate lethal dose, however, can be estimated from the study by Shimizu et al. (1996), which reports a fatal exposure of a 62.5 kg woman who intentionally consumed a pesticide formulation containing 75% DDVP and 25% xylene. While xylene is also a toxic agent, the oral LD_{50} for xylene in rodents is in the range of 3,500 to 8,600 mg/kg (ATSDR, 1995, p. 59). This is much greater than the reported LD_{50} values for DDVP in rodents – i.e., in the range of 25 to 300 mg/kg as summarized in Section 3.14. The amount of DDVP that the woman ingested is unclear. About 300 grams (300,000 mg) of DDVP were found in the stomach and Shimizu et al. (1996, p. 65) estimate that the woman probably absorbed about 1,000 mg/kg. Taking the estimated absorbed dose, a lethal dose for humans can be estimated at about 16 mg/kg [1,000 mg ÷ 62.5 kg]. This is not necessarily a minimum lethal dose – i.e., the individual might have died after ingesting a lesser amount of DDVP. Other reported poisoning cases involving DDVP (e.g., ATSDR 1997; WHO 1988) do not have sufficient information to estimate a minimum lethal dose for humans.

3.3.2.2. Acute Dermal – For short-term dermal exposure, the U.S. EPA (2000a) recommends an oral NOAEL of 0.1 mg/kg with a margin of exposure of 300 for residential exposure and 100 for occupational exposure. This would correspond to an acute RfD of 0.00033 mg/kg for residential exposures and 0.001 mg/kg for occupational exposures. The U.S. EPA (2000a) recommends using this value with dermal deposition data and an assumed dermal absorption fraction of 11%.

These values will not be used in the current risk assessment. Following the general approach used in other risk assessments prepared for USDA (SERA 2001), the absorbed doses estimated in Section 3.2.2.3 for workers and Section 3.2.3.2 for the general public will be used with the acute oral RfD of 0.0017 mg/kg/day. The general rationale for this approach is given in SERA (2001).

For DDVP in particular, the standard approach used in USDA risk assessments is necessary because the incidental or accidental handling of VaporTape strips does lead to estimates of dermal deposition.

3.3.2.3. Acute Inhalation – For short-term inhalation exposures, the U.S. EPA (2000a) recommends the same acute toxicity value used for dermal exposures. Given the extensive inhalation toxicity data available for DDVP, the rationale for this approach is unclear. The U.S. EPA (1994) has derived an inhalation RfC for DDVP of 0.0005 mg/m^3. This is based on an animal NOAEL of 0.05 mg/m^3 with a corresponding LOAEL of 0.48 mg/m^3 from a two year exposure study in rats. As noted below, this chronic RfD is not relevant to the current risk assessment because no chronic exposures are anticipated. In addition to this value recommended by EPA, ATSDR (1997) has recommended an acute minimum risk level (MRL) of 0.002 ppm for DDVP which corresponds to a concentration of about 0.018 mg/m^3 – i.e., 1 ppm = 9.04 mg/m^3. This value is intended to be applied to exposure periods of up to 14 days.

As detailed in Section 3.2.2.2, all exposures for workers are short-term. OSHA and NIOSH share responsibility for proposing exposure criteria to protect workers. OSHA provides regulatory enforcement (exposure standards) and NIOSH provides science based exposure criteria (NIOSH 2002). For DDVP, NIOSH recommends a time-weighted average exposure limit of 1 mg/m^3 and this value has been adopted by OSHA (NIOSH 2002). Another group involved in recommending criteria for occupational exposure is ACGIH (2004), which recommended a lower occupational exposure limit of 0.1 mg/m^3 (ACGIH 1991). This lower value appears to have been selected by ACGIH (1991) based on an unpublished report to the TLV committee that exposures to 1 mg/m^3 over the course of a workday resulted in an inhibition of plasma AChE of 20%-25% in a group of workers (ACGIH 1991, p. 446). The documentation for the TLV, however, does not suggest that any adverse health effects were observed. The lower and more protective value of 0.1 mg/m^3 is adopted in the current risk assessment for the protection of workers during inhalation exposures.

3.3.3. Chronic Exposures

The U.S. EPA (2002), ATSDR (1997), and WHO (1998) have all recommended various criteria for chronic exposure to DDVP by oral, dermal, and/or inhalation routes. Because none of the exposure scenarios in this risk assessment involve chronic or subchronic exposures, these recommendations are not considered in the current risk assessment. While the previous USDA risk assessment (USDA 1995a) considered the potential cancer risks associated with exposure to DDVP, this approach is not adopted in the current risk assessment. As discussed in Section 3.1.10, the recent re-evaluation of the cancer data on DDVP (U.S. EPA 2000a,b) has concluded that the data available on the carcinogenicity of DDVP is not sufficient for quantitative risk assessment.

3.4. RISK CHARACTERIZATION

3.4.1. Overview

The quantitative risk characterizations for workers and members of the general public are summarized in Table 3-3. This table is taken directly from Worksheet C02 and is included in the body of the risk assessment only for convenience.

In most cases, exposures to both workers and members of the general public should be negligible. If workers take prudent steps to limit both dermal and inhalation exposures, the likelihood of exposures to DDVP reaching a level of concern appears to be very low. Similarly, members of the general public should not be exposed to substantial amounts of DDVP. The DDVP is contained within a PVC strip to insure that the active ingredient is slowly released over a long period of time. The strip, in turn, is placed within a trap and the trap is placed in areas that will not be generally accessed except in the case of intentional tampering or trap monitoring.

Nonetheless, this risk assessment develops exposure scenarios for both workers and members of the general pubic that are intended to illustrate the potential effects of mishandling or tampering with DDVP strips. For workers, the greatest risks are associated with inhalation exposures from assembling the traps in enclosed and poorly ventilated spaces or transporting the traps in the passenger compartments of vehicles. These risks can be readily avoided. Dermal exposures can also lead to lesser but sill undesirable levels of exposure. For members of the general public, all of the exposure scenarios are accidental and some are extreme. The most likely of these is the accidental contamination of a small body of water. This scenario leads to exposures that are below the level of concern by a factor of about 25. If a child were to come into contact with a DDVP strip, however, both dermal and oral exposures could substantially exceed a level of concern. While such exposures should clearly be avoided, it does not seem likely that frank signs of toxicity would be observed. This is consistent with human experience in the use of DDVP resin strips.

3.4.2. Workers

The risk characterization for workers is highly dependant on how the worker handles the DDVP strip during assembly of the milk carton trap. If the trap is assembled outdoors and if the worker wears protective gloves during the assembly of the trap, both dermal and inhalation exposures as well as consequent risk should be negligible. Whether or not this is common practice is unclear. The MSDS states that gloves (vinyl, latex, or rubber) should be worn if the strip is handled for prolonged periods of time (Hercon 1993). The product label (Hercon 2004) indicates that hands should be washed thoroughly after handling the pest strip. In addition, the Gypsy Moth Program Manual (USDA 2001, p. E-6) recommends that workers "*use the outer package or rubber gloves to handle the insecticide strip. Handle the insecticide strip as little as possible*". If these recommendations are followed, direct dermal exposure to DDVP should be negligible.

If workers assemble traps in enclosed areas or do not use protective gloves during the assembly of traps or take other measures to prevent dermal exposure, it is plausible that exposures will exceed a level of concern. As summarized in Table 3-3, the potential for undesirable inhalation

exposures is substantial – i.e., risk quotients up to 18 – if the traps are assembled or transported in areas with poor or no ventilation. As discussed in Section 3.2.2.2 and detailed further in Appendix 1, these exposure assessments are based on a large number of site and situation specific factors – i.e., the volume of the room or area in which the strips are assembled or transported, the number of strips that are involved, and the ventilation rates of the area in which exposure occurs. Thus, if the pest strips are assembled indoors, it would be prudent to modify Worksheet A03a and ensure that the local conditions would likely lead to air concentrations that are below the ACGIH (1991) TLV of 0.1 mg/m^3.

It should be noted that the risk quotients associated with transport of the pest strips in the passenger compartment of a vehicle are substantially higher than risk quotients during assembly of the traps in a room. High ventilation rates – i.e., 3000 air turnovers per day or about 2 air turnovers per minute as detailed in Worksheet A03b – could probably be achieved in a vehicle by rolling down the window and this would reduce the inhalation exposure to below the level of concern. Nonetheless, transporting DDVP or any volatile neurotoxic agent in the passenger compartment of a vehicle is clearly imprudent and should be avoided.

Dermal exposure is of lesser and only modest concern based on the exposure assessments. As noted in Table 3-3, the acute RfD is modestly exceeded – i.e., a hazard quotient of 3 – at the upper range of estimated exposures if workers do not wear gloves . This risk quotient is associated with a dose of about 0.005 mg/kg bw. It seems unlikely that any adverse effects would be experienced at this dose level, which is a factor of 200 below the human NOAEL of 1 mg/kg [1 mg/kg ÷ 0.005 mg/kg = 200] and a factor of 3,200 below the lowest reported lethal dose in humans [16 mg/kg ÷ 0.005 mg/kg = 3200]. While there are uncertainties with the exposure assessment on which the risk quotient of 3 is based, contamination of the skin in workers not wearing gloves seems to be highly likely. As noted in the product label for the VaporTape II strip: "*After prolonged storage, a small amount of liquid may form on the strip*" (Hercon 2004). This liquid would presumably contain DDVP which would contaminate the surface of the exposed skin. It is also worth noting that the exposure assessment assumes that only the tips of the fingers are contaminated and that the duration of exposure is only 15 minutes to 1 hour. If the worker were to contaminate a greater area of the skin or to spend a longer period of time assembling the traps, the estimated doses would be greater.

3.4.3. General Public

The nature of risks to the general public is substantially different from those to workers. As detailed in the previous section, undesirable levels of exposure are plausible for workers if sensible measures are not taken to limit exposure. For members of the general public, essentially no significant exposures are plausible. The accidental contamination of a small pond with a pest strip (Worksheet B02) is probably the most likely exposure scenario. As indicated in Table 3-3, this exposure scenario leads to levels of risk that a very low – i.e., the highest hazard quotient is 0.04, below the level of concern by a factor of 25.

The probability of a child tampering with a trap is low because the traps will not generally be placed in areas that the general public will frequent and will be placed so that the traps are not easily accessible to children. Thus, the exposure scenarios involving a child either tampering with a trap or otherwise coming into direct contact with a DDVP strip appear to be highly unlikely. As illustrated in Table 3-3, dermal exposures would lead to risk quotients of up to 60. These exposures would be associated with doses of up to about 0.1 mg/kg (Worksheet B01b). This dose is below the lowest reported lethal dose in humans by a factor of about 160 [16 mg/kg ÷ 0.1 mg/kg], below the acute human NOAEL of 1 mg/kg by a factor of 10, and below the acute animal NOAEL of 0.5 mg/kg by a factor 5. Thus, while this type of exposure would be considered unacceptable, the plausibility of observing toxic effects seems remote.

The plausibility and consequences of oral exposures for a child tampering with a DDVP strip are very difficult to assess. The unpleasant taste and smell of the pest strip should help to decrease the amount of exposure; however, there are reported cases of child poisoning by pest strips containing DDVP, although none of the exposures have been fatal. Nonetheless, the oral exposure scenarios developed in this risk assessment lead to the highest risk quotients for DDVP, a central estimate of 97 with a range of 24 to 380 (Table 3-3 and Worksheet C02). These risk quotients are associated with doses of about 0.2 mg/kg with a range of about 0.04 mg/k to 0.6 mg/kg. As with the dermal exposures for a small child, these exposures should be clearly regarded as unacceptable. Nonetheless, it is not clear that any significant adverse effects would be observed since the dose estimates are below the human NOAEL of 1 mg/kg and the upper range of exposure is below the lowest reported lethal dose by a factor of over 25 [16 mg/kg ÷ 0.6 = 26.7]. Thus, while these exposure scenarios may be considered extreme and could warrant prompt medical attention as a precautionary measure, it is possible that no serious adverse effects would be observed. This risk characterization is consistent with the assessment of incidents involving exposures to DDVP resin strips – *"exposure to resin strips usually do not involve any significant acute symptoms that would require medical treatment"* (U.S. EPA 2000a, p. 26).

3.4.4. Sensitive Subgroups

Children are of primary concern to this risk assessment. As noted above, imprudent handling of a DDVP impregnated strip would most likely involve a child. In addition, very young children (that is, infants less than 6-months old) may be at special risk because they have incompletely developed AChE systems and immature livers (ATSDR 1993). Several other groups may be at special risk to all cholinesterase inhibiting compounds, including DDVP. A small proportion of the population has an atypical variant of plasma cholinesterase. This condition is known to make these individuals sensitive to succinylcholine and may make them more susceptible to exposure to DDVP and other AChE inhibitors. Other groups known to have low plasma AChE levels are long-distance runners, women in early stages of pregnancy, women using birth control pills, individuals with advanced liver disease, alcoholics, individuals with poor nutritional status, and individuals with skin diseases. Asthmatics may also be at special risk because DDVP may induce or exacerbate respiratory distress (ATSDR 1993).

3.4.5. Connected Actions

There are no data regarding the effects of exposure to DDVP combined with exposure to the other agents used to control the gypsy moth or the gypsy moth itself. Inhibition of AChE is the most sensitive effect of DDVP. This effect is not associated with exposure to the other control agents or exposure to the gypsy moth. Therefore, there is no plausible basis for assuming that the effects of exposure to DDVP and any or all of the other control agents or the gypsy moth will be additive.

Exposure to other compounds that inhibit AChE are likely to lead to an additive effect with DDVP. The most common examples include any other organophosphate or carbamate pesticides (ATSDR 1993; Gallo and Lawryk 1991). Thus, if members of the general public or workers use other organophosphate pesticides to the extent that AChE activity is substantially inhibited, they could be at increased risk if exposed to significant levels of DDVP.

No studies were located regarding toxicological interactions between Vaportape II and other chemicals. There are several studies regarding combined exposures to commercial grade DDVP and other chemicals, all of which involve animal exposure, and, in most cases, overtly neurotoxic doses of DDVP administered by acute injections. Of the few studies regarding oral or dermal exposure to DDVP, most involve acute durations of exposure and do not provide adequate evidence of toxicological interactions. Nevertheless, some of these studies are discussed here because they concern certain interactions that are generally associated with organophosphate insecticides as a class and because they are relevant to the issue of whether or not such interactions involving DDVP are plausible.

Phenothiazine-derived drugs such as chlorpromazine have been shown to enhance the toxicity of acutely administered organophosphate insecticides such as parathion (Calabrese 1991). The mechanism for this enhancement is not known and may involve altered metabolic activation or deactivation of the organophosphate. The interaction between topically applied DDVP/Crotoxyphos insecticide and orally administered phenothiazine anthelmintic has been studied to a limited extent in livestock, and no obvious interactions have been observed. A series of case studies were reported in which young cattle were treated with topical doses of various organophosphate insecticides at the end of a 30-day oral treatment with phenothiazine anthelmintic, followed by DDVP/Crotoxyphos insecticide 1 month later. There was no evidence of an interaction between the phenothiazine and DDVP/Crotoxyphos insecticide (Schlinke and Palmer 1973). In a more controlled study, lambs were treated orally with phenothiazine antihelmentic (12.5 g initially and 4 days later with 6.25 g every 3 days for nine treatments) or topical application of an emulsifiable mixture of 2.3% DDVP and 10% Crotoxyphos (1,550 mL of 0.25% emulsion sprayed every 2 weeks for three applications) or both. Erythrocyte acetylcholinesterase inhibition and clinical signs of acetylcholinesterase inhibition occurred within 40 minutes after each DDVP/Crotoxyphos mixture spray; the severity of the effects was not affected by the concurrent phenothiazine treatment (Mohammad and St. Omer 1983, 1985).

Because of their ability to inhibit acetylcholinesterase and thereby alter the metabolism and deactivation of acetylcholine, organophosphate insecticides are expected to interact with drugs that mimic the effect of acetylcholine (cholinergic drugs) or that block the effects of acetylcholine (anticholinergic drugs). In fact, the anticholinergic drug, atropine, is indicated for treatment of severe cholinergic symptoms of organophosphate insecticide toxicity. Because both cholinergic and anticholinergic drugs have many other uses, inadvertent interactions in which the organophosphate insecticide alters the effect of the drug also should be considered. Acute interactions of this type involving DDVP have been studied only to a limited extent in animal models of peripheral cholinergic control mechanisms. In one such study, the anticholinergic drug, atropine, was administered to dogs (0.022 mg/kg by intramuscular injection) 90 minutes after an acute oral dose of 60 mg/kg DDVP, and the heart rate was monitored for cholinergic (decreased rate) and anticholinergic (increased rate) effects. Although the DDVP dose alone had no effect on heart rate, it did attenuate the acceleration of the heart rate caused by atropine. The DDVP dose decreased plasma and erythrocyte cholinesterase by approximately 50% (Dellinger et al. 1987). This study suggests that interactions in which DDVP affects the actions of anticholinergic drugs (for example, atropine, scopolamine, belladonna alkaloids) are plausible; however, there is no evidence of such interactions in humans.

Chemicals that inhibit carboxyesterases such as the non-organophosphate insecticide, triorthotolyl phosphate (TOTP), have been shown to enhance the toxicity of certain organophosphate insecticides. Inhibition of carboxyesterases may be a mechanism by which certain organophosphate insecticides act synergistically (Calabrese 1991). The significance of this interaction mechanism to DDVP toxicity has not been thoroughly investigated. In a study using mice, an acute intraperitoneal dose of TOTP 3 days before DDVP treatment enhanced the toxicity of an acute intraperitoneal dose of either malaoxon or paraoxon but did not alter the toxicity of an intraperitoneal dose of DDVP. Dieldrin, administered orally 4 days before sacrifice, increased liver carboxyesterase activity but had no effect on the toxicity of subsequently administered DDVP (Ehrich and Cohen 1977). This study suggests that carboxyesterase inhibitors may have a more significant effect on malaoxon and paraoxon toxicity than on DDVP toxicity.

The interaction of DDVP with other commonly occurring chemicals in the environment has not been well studied. In rats, pretreatment with acetaminophen, a common analgesic, had no effect on the acute toxicity of DDVP (Costa and Murphy 1984).

Toxicological interactions of DDVP have not been studied extensively or well enough to be of use in quantitative risk assessment. The few studies described here suggest that certain interactions typical of the organophosphate insecticides as a class (for example, anticholinergic agents) are plausible for DDVP. Nevertheless, there is no evidence that such interactions actually occur in humans. Furthermore, the studies regarding those kinds of interactions in animals have examined single exposures and have focused only on the acute anticholinesterase activity as the toxic endpoint (usually assessed by measurements of plasma or blood cholinesterase or cholinergic symptoms). There need to be more complete interaction bioassays

that examine multiple dose levels and durations, and more complete assessments of toxicity if risks related to possible interactions are to be assessed.

3.4.6. Cumulative Effects

Cumulative effects associated with DDVP exposures might be associated with repeated exposures during a single season or repeated exposures over several seasons. For the general public, the only substantial exposures will occur from tampering with traps containing DDVP. Such incidents have not been reported despite the long use of DDVP in traps for the gypsy moth as well as other species. These scenarios are considered in this risk assessment as accidental exposures, which occur infrequently. Consequently, it does not seem reasonable to expect that the same person will be involved repeatedly in such unusual exposures.

Workers, on the other hand, may be exposed repeatedly to DDVP if they are involved in the assembly and placement of traps over a period of several weeks. Such exposures, however, are encompassed by the current risk assessment. For inhalation exposures, the risk is characterized using the TLV (ACGIH 1991). The TLV is intended to be protective of exposures that occur during a typical career (for example, 8 hours/day, 5 days/week, for 20 years).

For some organophosphates, concern about cumulative effects is diminished because studies have demonstrated tolerance to repeated exposures (Gallo and Lawryk 1991). This tolerance has not been demonstrated for exposure to DDVP. As is true for exposures involving the general public, concern for repeated exposures is diminished because, under normal handling conditions, substantial levels of exposure are not anticipated.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview

As described in Section 3.1.2., DDVP is an organophosphate insecticide. DDVP inhibits acetylcholinesterase (AChE) activity, resulting in overstimulation of cholinergic neurons. Inhibition of this enzyme in mammalian systems produces a variety of systemic effects, including salivation, urination, lacrimation, convulsions, increased bronchial secretions, respiratory depression, and even death. DDVP is readily absorbed by the oral, dermal, and inhalation routes of exposure. Because the target enzyme (cholinesterase) for DDVP is common to mammals, fish, fowl, and insects, toxicity due to DDVP exposure can result in all of these species. By contrast, DDVP exhibits low toxicity to plants.

The available data suggest that invertebrates are more sensitive to DDVP than other organisms. For example, the oral LD_{50} in honey bees is 0.29 µg/g bee, and the topical LD_{50} is 0.65 µg/g bee. DDVP is also toxic to birds with an oral LD_{50} value of < 10 mg/kg for the most sensitive species. Short-term repeat dose studies in mammals found that oral exposures to doses below about 0.5 mg/kg-day or inhalation exposures to 1–2 mg/m³ generally do not result in adverse effects.

Aquatic animals are also sensitive to DDVP and, as with terrestrial animals, invertebrates may be more sensitive than vertebrates. The lowest reported LC_{50} value in fish is approximately 0.2 mg/L. Some aquatic invertebrates are much more sensitive to DDVP than fish. For daphnids, the most sensitive group of invertebrate species, reported EC_{50} values range from 0.00007 mg/L to 0.00028 mg/L.

The majority of the toxicity data in ecological receptors is limited to free DDVP, rather than a slow-release formulation such as the Vaportape II product used in USDA programs for control of the gypsy moth. Hence, the toxicity values reported for indicator species will likely be conservative (i.e., suggest greater toxicity) as compared to Vaportape II. U.S. EPA has assessed the ecological effects of DDVP; however, the exposures assessed by U.S. EPA are not specific to formulations where DDVP is encapsulated in PVC resin. In general, aside from those organisms that enter the milk carton trap or those that remove the strip from the trap, toxicity resulting from exposure of ecological receptors to DDVP in Vaportape II milk carton traps is not likely.

4.1.2. Toxicity to Terrestrial Organisms.

4.1.2.1. Mammals – As summarized in Section 3.1, the database includes a number of toxicity studies in experimental mammals. The principal adverse effects of DDVP exposure are directly related to inhibition of cholinesterase (the mode of action for DDVP). Inhibition of this enzyme in mammalian systems produces a variety of systemic effects (Table 3-1). The nature and magnitude of the toxicity produced by a given exposure to DDVP by any route are directly related to the dose and rate at which the exposure occurs. In USDA programs for the control of the gypsy moth, the use of milk carton traps employing slow-release of DDVP from PVC strips essentially precludes rapid exposures to high doses of DDVP. As described in Section 3.1.4,

short-term animal studies have shown that oral exposures to free DDVP below about 0.5 mg/kg-day (or inhalation exposures to 1–2 mg/m³) do not result in meaningful reductions in cholinesterase activity. Experiments in laboratory mammals that were exposed to DDVP during pregnancy (by oral or inhalation route) did not show any effect on fertility or health of the offspring, even at levels that produced maternal toxicity (see Section 3.1.9).

Dietary administration of DDVP (free and encapsulated in PVC resin pellets) has been used as a veterinary anthelminthic agent in a variety of species, including dogs (Batte et al. 1966; Batte et al. 1967), pigs (Batte et al. 1965; Bris et al. 1968; Stanton et al. 1979; Todd 1967), horses (Himes et al. 1967), sheep (Bris et al. 1968), cattle (Bris et al. 1968), dromedary camels (Wallach and Frueh 1968), and non-human primates (Wallach and Frueh 1968). In general, oral administration of DDVP produced no signs of organophosphate poisoning at doses that were effective at reducing intestinal parasites (Wallach and Frueh 1968). For example, two consecutive days of dosing at 2.3 in camels or 1.7 mg/kg in non-human primates, respectively, was well tolerated by the animals despite debilitating intestinal infection (Wallach and Fueh 1968). In cows, Lloyd and Matthysse (1971) reported that diets containing DDVP (in PVC pellets) at doses 1.3, 1.8, or 2.3 mg/kg bw for 14 days produced no adverse effect on milk production (no other effects were reported). No DDVP was found in the milk at 1, 3, 7, 10 or 14 days. Free DDVP – i.e., not encapsulated in a PVC resin – produced severe inhibition of cholinesterase activity at a dose of 4.5 mg/kg (Tracey et al 1960).

As discussed in Section 3.1.4, the effect of PVC encapsulation on the toxicity of DDVP has been quantified in parallel assays (Stanton et al. 1979), in which DDVP (undiluted technical grade) and DDVP (impregnated in PVC) were administered to groups of young swine. For the technical grade liquid formulation, the LD_{50} was 157 (113–227) mg/kg and the NOAEL based on lethality was 56 mg/kg. For the PVC formulation, no deaths occurred at any doses including 1,000 mg/kg, the highest dose tested.

As discussed in Section 3.1.16, simultaneous exposure to DDVP and another cholinesterase inhibitor (e.g., organophosphate or carbamate insecticides) or a cholinomimetic agent (e.g., pilocarpine and carbachol) would likely enhance the cholinergic toxicity produced by DDVP. This is the major toxicologic interaction for DDVP. In addition, Short et al. (1971) also reported that DDVP exposure in combination with the muscle relaxant succinylcholine can produce cardiac arrythmias, apnea, and death in Shetland ponies depending on the degree of cholinesterase inhibition.

4.1.2.2. Birds – The acute oral LD_{50} in birds ranges from 6.5–24 mg/kg (WHO 1989, Hudson et al. 1984, Grimes and Aber 1988). As in mammals, the signs of DDVP intoxication in birds are typical of organophosphorus poisoning (e.g., tremors, and convulsions) and usually appear shortly after dosing. At lethal doses, death occurs within 1 hour, with survivors recovering completely within 24 h after dosing (WHO 1989). Tucker and Crabtree (1970) found various internal hemorrhages at autopsy in sacrificed pheasants and mallard ducks that survived acute high dose exposures.

The data from unpublished egg production and hatchability studies suggests that mallard ducks are more sensitive to DDVP than northern bobwhite quail. In mallard ducks, 20 weeks of dietary exposure identified a NOEC of 5 ppm and a LOAEL of 15 ppm based on number of eggs laid, eggshell thickness, number of viable embryos and number of live 3-week embryos (Redgrave and Mansell 1997). Cameron (1996) reported no effect on bobwhite quail reproduction following dietary exposure to DDVP at concentrations of 12 or 30 ppm for 20 weeks. At 100 ppm, however, statistically significant reductions in the number of eggs laid, viable embryos, live 3-week embryos, and survivors at 14 days. The short-term dietary LD_{50} in birds (5 days of exposure followed by three days of untreated diet) ranged from 300 ppm in Japanese quail to 5000 ppm in mallard ducks (Hill et al. 1975). Using chick and duck eggs, injections with DDVP at various incubation stages revealed that the LD_{50} values for these avian species at the mid-incubation stage were comparable to the rodent oral LD_{50} values (i.e., >50 mg/kg) (Khera and Lyon 1968).

Five days of continuous exposure of canaries, Indian finches, and budgerigars to DDVP vapor at 0.14 mg/m³ reduced cholinesterase activity, but produced no overt signs of organophosphate intoxication (Brown et al. 1968, as cited by WHO 1989).

It is important to note that the LD_{50} values reported from these studies are derived from the active ingredient, DDVP, in free form. Encapsulation in PVC resin (such as Vaportape II used in milk carton traps) would be expected to slow the release of DDVP, thereby reducing the acute toxicity and increasing the LD_{50} values (Section 3.1.4). No published data are available concerning the acute toxicity of DDVP encased in PVC resin in birds.

4.1.2.3. Terrestrial Invertebrates – In general DDVP is highly toxic to invertebrates with effect levels for honey bees below 1 µg/g bee. In laboratory studies of honey bees, Atkins et al. (1973) found an LD_{50} of 0.495 µg/bee in 48 h (topical application of dust; 26.7 °C with a relative humidity 65%). Beran (1979) reported an oral LD_{50} of 0.29 µg/g body weight and a topical LD_{50} of 0.65 µg/g body weight.

A variety of other studies are available; however, they are not reported in sufficient detail to provide quantitative estimates of exposures. Nevertheless, these studies support the conclusion that invertebrates are highly susceptible to the toxic effects of DDVP. Following the exposure of honeycombs to DDVP vapor emanating from DDVP resin strips for 4 months, the combs absorbed the insecticide and were toxic to bees for approximately one month after exposure. Contamination of the bees appeared to be by inhalation rather than direct contact (Clinch 1970). Consumption of mulberry leaves sprayed with 1.56–6.25 mg/L DDVP produced 50% mortality in silkworm larvae after 4 hours of feeding (Aratake and Kayamura 1973). No adverse effects were observed on the hatchability and general condition of silkworm larvae hatched in the generation following feeding of mulberry leaves pre-treated with 3 mg/kg DDVP of leaf to adults (Yamanoi 1980).

4.1.2.4. Terrestrial Plants (Macrophytes) – Neither the published literature nor the review documents include data regarding the phytotoxicity of DDVP. Given the mode of action of DDVP, the U.S. EPA (1999a) has determined that toxicity testing in plants is not required for registration.

4.1.2.5. Terrestrial Microorganisms – WHO (1989) reported that the effect of DDVP on microorganisms is variable and species dependent. Certain microorganisms are able to metabolize DDVP, but DDVP may interfere with the endogenous oxidative metabolism of the organism. In certain organisms DDVP inhibits growth, while in others it has no influence or may stimulate growth. The above effects have been seen over a concentration range of 0.1–100 mg/L (Lieberman and Alexander 1981).

As noted earlier, the LD_{50} values reported from these studies are derived from the active ingredient, DDVP, in free form. Encapsulation in PVC resin (such as Vaportape II used by the Forest Service in milk carton traps) would be expected to slow the release of DDVP, thereby reducing the acute toxicity and increasing the LD_{50} values. No published data are available concerning the acute toxicity of DDVP encased in PVC resin in terrestrial microorganisms.

4.1.2.6. Terrestrial Field Studies – No terrestrial field studies on the effects of free DDVP or DDVP in PVC resin were located. Whitehead (1971) has advised caution in the use and handling of DDVP, where birds might be exposed because of their particular sensitivity to the toxic effects of organophosphate poisoning. In the case of the USDA programs involving the use of DDVP in traps, however, the probability of widespread contamination of soil or aquatic ecosystems is very low because a small amount of DDVP (590 mg) is used in the Vaportape II trap and because the DDVP is released slowly from the PVC resin.

4.1.3. Aquatic Organisms.

4.1.3.1. Fish – DDVP is classified as highly toxic to both freshwater and estuarine fish (U.S. EPA 1999a). In freshwater fish, reported 96-h LC_{50} values range from about 0.2 mg/L for lake trout or cutthroat trout and 12 mg/L for fathead minnows (U.S. EPA 1999a, p. 12). In estuarine fish, 96-h LC_{50} values range from 0.23–14.4 mg/L for striped mullet and mummichog, respectively (U.S. EPA 1999a, p. 12). Sublethal effects – i.e., brain and liver cholinesterase inhibition – have been reported in fish at doses of 0.25–1.25 mg/L, but cholinesterase activity recovered when the fish were returned to clean water (WHO 1989). The acute toxicity of DDVP in cutthroat trout or lake trout was not altered by variations in water hardness from 44 to 162 mg/L or at pH 6 to 9 (Johnson and Finley 1980).

Studies of sublethal effects in fish, most involving exposure periods of about 30 days, have demonstrated that exposure to ≤ 1 mg/L DDVP may produce changes in respiratory rates, serum and liver enzyme activity (aside from cholinesterase), lipid and carbohydrate metabolism, and hemoglobin and clotting time (WHO 1989). From these reports of adverse effects in fish, WHO (1989) derived an NOEC of 0.03 mg/L.

Only unpublished studies submitted to U.S. EPA were located regarding the chronic toxicity of DDVP in fish. These studies are all summarized in U.S. EPA (1999a). A NOEC of 0.0052 mg/L was reported for rainbow trout with a corresponding LOAEL of 0.0101 mg/L for a reduction in larval survival. Another study found that 0.96 mg/L produced no effects on fry of sheepshead minnow, whereas 1.84 mg/L produced statistically significant reductions in fry survival and length. As discussed in Section 3.1.7., *in vitro* studies on cells from embryonic renal tissue of carp demonstrated a dose-related decrease in lymphocyte proliferation and myeloid cell respiratory burst activities, both of which indicate immunosuppression; however, no effects on antibody production were noted in an *in vivo* study of carp cells (Dunier et al. 1991). The authors concluded that the results suggest that chronic exposure to DDVP may impair the immune system of fish.

4.1.3.2. Amphibians – Neither the published literature nor the review documents include data regarding the toxicity of DDVP to amphibians.

4.1.3.3. Aquatic Invertebrates – In general, invertebrates tend to be more sensitive to the toxic effects of DDVP than fish. Whereas the lowest reported LC_{50} value reported in fish is 0.183 mg/L (the value for lake trout reported by U.S. EPA 1999a, p. 12), the lowest comparable value reported for aquatic invertebrates is 0.00007 mg/L (the 48-hour EC_{50} value for *Daphnia pulex* reported by U.S. EPA 1999a, p. 13). Based on these measures, aquatic invertebrates appear to be more sensitive than fish by a factor of over 2500 [0.183 mg/L ÷ 0.00007 mg/L = 2614]. WHO (1989) reports that the acute toxicity of DDVP to aquatic insects (stone fly) and estuarine crustaceans (hermit crab) is also extremely high (96-hour LC_{50} values ranging from 0.0001–0.045 mg/L, respectively).

As with the data on fish, some of the more important studies are unpublished and have been submitted to U.S. EPA for the registration of various uses of DDVP (U.S. EPA 1999a). As summarized by U.S. EPA (1999a), the 48-hour EC_{50} values in two species of water flea range from 0.00007 mg/L to 0.00028 mg/L. In an unpublished 21-day study in daphnids, the NOEC and LOEC are 0.0000058 mg/L and 0.0000122 mg/L, respectively.

Not all species of aquatic invertebrates, however, are this sensitive. The most remarkable exception to the sensitivity of aquatic invertebrates to DDVP is the freshwater snail; Jonnalagadda and Rao (1996) reported a 96-hour LC_{50} of approximately 21 mg/L in this species. Exposure of prawns to DDVP concentrations of 0.31 or 0.62 mg/L for 96 hours produced a decrease in hepatic glycogen and an increase in the blood glucose level (Omkar and Shukla 1984).

Forget et al. (1998) report static 96-hour LC_{50} values for copepods ranging from 0.00092–0.0046 mg/L (different sensitivity depending on life stage). Treatment of eutrophic carp ponds with 0.325 mg/L DDVP killed *Cladocera* (predominantly *Bosmina* and *Daphnia* species) and decreased cyclopods (mainly *Cyclops*). These reductions were offset by increased development of rotifers (mainly *Polyarthra* and *Brachionus* species) and phytoplankton (mainly *Scenedesmus*

and *Pediastrum* species), so that the total plankton biomass changed only slightly (Grahl et al. 1981).

4.1.3.4. Aquatic Plants – The database for DDVP does not contain many reports of its toxicity in aquatic plants. In an unpublished report cited by U.S. EPA 1999a), EC_{50} values >100 ppm are reported for green algae, 14 ppm for algae (NOS), and 17-28 ppm for marine diatoms. Butler (1977) reported that 3.5 mg/L DDVP produces 50% growth inhibition of *Euglena gracilis* (algae).

4.1.3.5. Other Aquatic Microorganisms – Neither the published literature nor the review documents include data regarding the toxicity of DDVP to other aquatic microorganisms.

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview

As in the human health risk assessment, exposure of terrestrial mammals to DDVP from the VaporTape strips used in milk carton traps is likely to be negligible under most circumstances. Nonetheless, it is conceivable that some mammals such as racoons or bears could easily access and tamper with the milk carton trap. Depending on the proportion of the DDVP strip that is consumed, doses (as DDVP in the PVC strip) are estimated to range from 10.5 mg/kg (10% of strip) to 105 mg/kg (100% of strip) and the central estimate is taken as 31.6 mg/kg (30% of strip). In addition, contamination of water with a pest strip is plausible, although probably rare, and is considered in a manner similar to the corresponding scenario in the human health risk assessment (Section 3.2.3.4). This scenario is based on the consumption of contaminated water by a small mammal and the dose to the animal is estimated at about 0.00003 mg/kg with a range of 0.000009 mg/kg to 0.00009 mg/kg. Other exposure scenarios for terrestrial vertebrates, while possible, seem far less plausible and are not considered quantitatively. No quantitative exposure assessments for terrestrial invertebrates are developed because the milk carton trap will attract only male gypsy moths because of the pheromone bait in the milk carton trap. Nontarget insects that incidentally enter the trap are likely to be killed by exposure to the DDVP vapor. Exposures to aquatic species are based on the same water concentrations used for terrestrial species: 0.000177 mg/L with a range of 0.000059 mg/L to 0.00059 mg/L.

4.2.2. Terrestrial Vertebrates

4.2.2.1. Oral Exposure to DDVP Strip – For the exposure of a young child discussed in Section 3.2.3.3, only sucking on the strip rather than ingestion of all or part of the strip is considered. Various species of wildlife, however, are probably capable of consuming all or part of a pest strip. For the current risk assessment, it will be assumed that a racoon tampers with a milk carton trap and consumes part or all of the strip – i.e., 590 mg of DDVP in the PVC formulation. Taking a body weight of about 5.6 kg for an adult racoon (the average of the values reported by U.S. EPA/ORD 1993, p. 2-236) and assuming that the animal consumes between 10% and 100% of the strip with a central value of 30%, the dose to the racoon would be about 31.6 mg/kg with a range of 10.5 mg/kg to 105 mg/kg (Worksheet D01).

4.2.2.2. Oral Exposure to Water Contaminated with DDVP – Estimated concentrations of DDVP in water are identical to those used in the human health risk assessment (Worksheet B02) and involve the accidental contamination of a small pond with a DDVP-PVC strip. The only major differences in this scenario compared to the scenario in the human health risk assessment involve the weight of the animal and the amount of water consumed. There are well-established relationships between body weight and water consumption across a wide range of mammalian species (e.g., U.S. EPA/ORD 1993). These relationships are used to estimate the amount of water that a 20 g mammal would consume in one day (Worksheet D02). Unlike the human health risk assessment, estimates of the variability of water consumption are not available. Thus, for this acute scenario, the only factor affecting the variability of the ingested dose estimates is the amount of DDVP that might be released in one day. These amounts are discussed in Section

3.2.3.4 and are used in Worksheet D02. As indicated in Worksheet D02, the central estimate of the dose is about 0.00003 mg/kg with a range of 0.000009 mg/kg to 0.00009 mg/kg.

4.2.3. Terrestrial Invertebrates

As in the previous risk assessment (USDA 1995b), quantitative exposure assessments for terrestrial invertebrates are not considered. The only terrestrial invertebrates that are likely to come into close contact with the DDVP strip are male gypsy moths, which will be attracted by the disparlure in the trap, or carnivorous wasps and hornets that may enter the trap to feed on dead and dying gypsy moths. Other insects and perhaps other invertebrates such as spiders might incidentally enter the milk carton traps. Because DDVP in a non-specific insecticide, nontarget invertebrates would likely be killed by exposure to the DDVP vapor within the trap.

4.2.4. Aquatic Species

The exposure assessment for aquatic species is based on concentrations of DDVP in water that are identical to the concentrations used in the human health risk assessment (Worksheet B02) and the exposure assessment for a small mammal drinking contaminated water (Worksheet D02). As indicated in these worksheets, the central estimate of the concentration of DDVP in the pond is 0.000177 mg/L with a range of 0.000059 mg/L to 0.00059 mg/L.

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Overview

Given the limited nature of the use of DDVP in programs to control the gypsy moth and consequent limited number of exposure assessments, the dose-response assessment for DDVP is relatively simple. For terrestrial mammals, a value of 240 mg/kg from a study using DDVP in a PVC formulation is used for direct exposure to the DDVP-PVC strip – i.e., a raccoon tampering with a milk carton trap and consuming all or part of the DDVP strip. At the dose of 240 mg/kg, no mortality or frank signs of AChE inhibition were observed. For the contaminated water scenario, the NOAEL of 0.5 mg/kg from a study involving exposure to free or unformulated DDVP is used. This NOAEL is from the study that forms the basis for the acute RfD used in the human health risk assessment. Although DDVP is classified as highly toxic to fish, the estimated levels of acute exposure for fish are far below the 30-day NOEC of 0.03 mg/L. Thus, this value is used for all fish and no attempt is made to consider differences in sensitivity among fish. A somewhat different approach is taken with aquatic invertebrates, some of which are more sensitive to DDVP than fish by a factor of over 2500. Risks to sensitive species of aquatic invertebrates – i.e., daphnids and other small arthropods – are characterized based on the lowest reported LC_{50} value, 0.00007 mg/L from a 48-hour bioassay in *Daphnia pulex*. Some other groups of aquatic invertebrates, such as snails, appear to be much less sensitive than small arthropods. Risks to such tolerant species are based on a LC_{50} value of 21 mg/L in a freshwater snail.

4.3.2. Toxicity to Terrestrial Organisms

Two different types of exposure assessments are given for terrestrial vertebrates: direct consumption of all or part of the DDVP-PVC stip (Section 4.2.2.1) and consumption of water contaminated with DDVP (4.2.2.2). The former scenario involves exposure to the formulated DDVP and the latter exposure scenario involves exposure to unformulated or free DDVP. For the exposure assessment involving direct consumption of the DDVP-PVC strip, the dose of 240 mg/kg for neurotoxicity from the study by Stanton et al. (1979) will be used to characterize risk. No mortality or frank signs of AChE inhibition were observed at this dose. For exposure to free DDVP in water, the NOAEL of 0.5 mg/kg for changes in AChE activity and other signs of neurotoxicity will be used to characterize risk. This is the NOAEL selected by the U.S. EPA (2000a) as the basis for the acute oral RfD for DDVP. As indicated in Section 4.4., these two NOAEL values are substantially below the corresponding exposure levels. Thus, elaboration of the dose-response assessment is not necessary.

4.3.3. Aquatic Organisms

4.3.3.1. Fish – The U.S. EPA typically uses LC_{50} values as benchmark doses for developing acute hazard quotients and the most sensitive LC_{50} of 0.183 mg/L was used by U.S. EPA in it's ecological risk assessment for DDVP (U.S. EPA 1999a, p. 29). USDA risk assessments typically prefer to use NOEC (no observed effect concentrations) when such data are available. As discussed in Section 4.1.3.1, WHO (1989) has identified an NOEC of 0.03 mg/L from studies involving exposure periods of about 30 days. This NOEC will be adopted in the current risk assessment. While the application of a 30-day NOEC to the acute and much shorter term

exposures considered in this risk assessment is likely to be over-protective, this has no impact on the characterization of risk because the anticipated levels of acute exposure are substantially below this NOEC. Also because this conservative NOEC value is below a level of concern, separate assessments are not made for sensitive and tolerant species of fish. This is discussed further in Section 4.4.

4.3.3.2. Aquatic Invertebrates **–** As noted in Section 4.1.3.3, some aquatic invertebrates are much more sensitive to DDVP than fish. Based on the lowest reported LC_{50} values in fish and invertebrates, some aquatic invertebrates are more sensitive than fish by a factor of over 2500. There is, however, a very wide range of tolerances in aquatic invertebrates. The lowest reported LC_{50} value is 0.00007 mg/L. This is a 48-hour LC_{50} value in *Daphnia pulex* reported by U.S. EPA (1999a, p. 13). A NOEC value is not reported by U.S. EPA (1999a). Thus, the LC_{50} 0.00007 mg/L is used directly in the risk characterization for sensitive aquatic invertebrates. As also noted in Section 4.1.3.3, the sensitivity of aquatic invertebrates to DDVP is highly variable. The least sensitive group of species appears to be aquatic snails, with a reported 96-hour LC_{50} of 21 mg/L (Jonnalagadda and Rao 1996). This value will be used to characterize risks in tolerant aquatic invertebrates.

4.4. RISK CHARACTERIZATION

4.4.1. Overview

As with the human health risk assessment, it is anticipated that typical exposures and consequent risks to nontarget species should be negligible. As with the human health risk assessment, it is anticipated that typical exposures and consequent risks to most nontarget species should be negligible. The containment of the DDVP within a slow release PVC strip combined with the target specific nature of pheromone baited traps should reduce the risks of inadvertent effects in non-target species. Other insects and arthropods that may inadvertently enter the trap will probably be killed by DDVP vapor. While such inadvertent contact may occur, it is not likely to impact substantial numbers of nontarget insects or arthropods.

Because of the limited use of DDVP, a relatively small number of exposure scenarios – all of which might be considered accidental or incidental – are developed. For terrestrial mammals, contact with the pest strip could occur by an animal directly tampering with a trap or by an animal consuming water that had been accidentally contaminated with a DDVP strip. Adverse effects would not be expected in either case. In the case of accidental contamination of a small body of water with a DDVP strip, concentrations of DDVP in the water would be below the level of concern for fish by factors of about 50 to 500. Some aquatic invertebrates, however, might be affected. For the most sensitive species of aquatic invertebrates – i.e., small aquatic arthropods such as daphnids – exposures could substantially exceed laboratory LC_{50} values by factors of up to about 8. Exposures to tolerant aquatic invertebrates – such as snails – would be below a level of concern by a substantial margin – i.e., factors of about 30,000 to 300,000. The exposure assessments that serve as the bases for these risk characterizations are highly dependent on specific conditions – i.e., how much DDVP was in the strip at the time that the contamination occurred and the size of the body of water that was contaminated.

4.4.2. Terrestrial Organisms

There is no indication that adverse effects in terrestrial vertebrates are likely. This assessment is based on the exposure scenarios for a relatively small mammal – i.e., a raccoon – consuming all or part of a DDVP-PVC strip as well as a very small mammal consuming water that had been contaminated with a pest strip.

The former scenario, direct consumption, may be plausible but is clearly extreme. The upper range of the exposure assessment assumes that the animal consumes the entire strip with a resulting dose of about 100 mg/kg (Section 4.2.2.1). The assessment of risk is based on a controlled laboratory study using a DDVP-PVC formulation in which no mortality was observed at 1,000 mg/kg and no signs of AChE inhibition were apparent at 240 mg/kg (Section 4.3.2). The dose of 100 mg/kg associated with upper range of the hazard quotient, 0.4, is below the the NOAEL by a factor 2.5.

The scenario for the consumption of contaminated water is based the assumption that a fresh DDVP strip inadvertently contaminates a small pond and, at the upper range of the estimated dose, the further assumption that all of the DDVP in the strip leaches into the water (Section

4.2.2.2 and Worksheets D02). The estimated dose is probably higher and perhaps much higher than what might actually occur because degradation of the DDVP in water is not considered. Even with these highly protective assumptions, the upper range of the risk quotient is only 0.0002 – i.e., below the level of concern (1) by a factor of 5,000. Thus, there is no plausible basis for asserting that adverse effects are likely.

No quantitative risk characterization is presented for terrestrial invertebrates. This approach is taken because there is no reason to anticipate that significant exposures to nontarget invertebrates are likely. It is possible that some insects and perhaps other arthropods could inadvertently enter a milk carton trap. In such a case, it is likely that the nontarget organisms would be killed by the DDVP vapor. While this is the intended effect in the target species, the gypsy moth, the efficacy of the traps is dependant on the use of another agent, disparlure, that serves as an attractant to male gypsy moths. As discussed in the risk assessment for disparlure, this attractant is highly specific to the gypsy moth and will not attract other species. Thus, the numbers of nontarget species that might be killed by inadvertently entering the traps is likely to be small and inconsequential.

4.4.3. Aquatic Organisms

4.4.3.1. Fish – There is no indication that fish are likely to be adversely affected by the use of DDVP in PVC strips. The exposure assessment for fish (Section 4.2.4) is based on the same very conservative exposure assessment used for mammals – i.e., the concentrations in water are likely to be over-estimated. The dose-response assessment is based on a 30-day NOEC for sublethal effects. The resulting risk quotients – i.e., 0.002 to 0.2 – are below the level of concern by factors of 50 to 500.

4.4.3.2. Aquatic Invertebrates – As discussed in Section 4.3.3.2, some aquatic invertebrates are much more sensitive to DDVP than fish and this difference in sensitivity impacts the characterization of risk. Based on the same conservative exposure assessment used for both fish and terrestrial vertebrates, some sensitive aquatic invertebrates could be adversely affected by DDVP contamination of water. As in the other exposure assessments involving contaminated water, this exposure scenario should be regarded as accidental rather than routine. In other words, under normal circumstances, water contamination from DDVP strips will be negligible and this is consistent with the conclusions reached by U.S. EPA (1999a, p. 25). Nonetheless, based on the modeled concentrations in the event of the accidental deposition of a strip containing 590 mg of DDVP into a small pond, concentrations of DDVP in the water would reach or substantially exceed the LC_{50} value for sensitive invertebrates and substantial mortality in sensitive invertebrates could occur.

The actual extent of mortality would depend on the rate at which DDVP is released from the strip, the degree of mixing that occurs in the water, and the rate of breakdown and dissipation of DDVP. These processes cannot be generically modeled but the conservative exposure assessment used to estimate concentrations in water suggests that adverse effects in sensitive aquatic invertebrates are plausible. No effects are likely in less sensitive aquatic invertebrates

such as aquatic snails. As discussed in Section 3.2.3.4, the hydrolysis of DDVP in water is rapid and it is likely that the estimates of adverse effects in some aquatic invertebrates would apply to only a very limited area near the pest strip rather than to the larger area of the body of water that is contaminated.

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Table 2-1: Selected physical and chemical properties of DDVP

Synonyms and trade names	SD 1750; Astrobot; Atgard; Canogard; Dedevap; Dichlorman; Dichlorophos; Dichlorvos; Divipan; Equigard; Equigel; Estrosol; Herkol; Nogos; Nuvan: Task; Vapona; Verdisol (Budavari 1989)
U.S. EPA Reg. No.	8730-50 (Hercon 2004)
CAS number	62-73-7 (ARS/PPD 1995; Meylan and Howard 2000)
Molecular weight	220.98 (Budavari 1989)
Molecular formula	$C_4H_7Cl_2O_4P$ (ARS/PPD 1995; Budavari 1989; Meylan and Howard 2000)
SMILES Notation	O=P(OC)(OC)OC=C(CL)CL (Meylan and Howard 2000)
Appearance/state, ambient	Liquid (ARS/PPD 1995; Budavari 1989)
mg/L to ppm conversion for air concentrations	1 ppm = 9.04 mg/m^3 (NOISH 2002) 1 mg/m^3 = 0.11 ppm
Boiling point	120°C at 14 mm Hg (ARS/PPD 1995) 251.76 °C (Meylan and Howard 2000)
Vapor pressure	1.2×10^{-2} mm Hg (Budavari 1989) 1,600 mPa (ARS/PPD 1995)
Water solubility (mg/L)	10,000 (Budavari 1989) 8,000 (ARS/PPD 1995)
Specific gravity	1.44 (Shell Chemical Company 1972)
log K_{ow}	1.40-2.29 (ARS/PPD 1995) [i.e., $K_{ow} = 10^{1.4} = 25.1$] 0.60 (estimated) (Meylan and Howard 2000) 1.47 (experimental) (Meylan and Howard 2000; U.S. EPA 1992)
Henry's law constant	0.044 Pa m^3/mole at 20°C (ARS/PPD 1995) 8.58E-007 atm-m^3/mole (Meylan and Howard 2000)
Koc	40.2 (Meylan and Howard 2000)
BCF	0.4486 (Meylan and Howard 2000)
Hydrolysis half-time (days)	0.022 to 0.347 (ARS/PPD 1995)
Aqueous photolysis halftime (days)	2.295 (ARS/PPD 1995)

Table 3-1. Common effects of acetylcholinesterase inhibition [a]

System	Receptor Type	Organ	Action	Manifestation
Parasympathetic	Muscarinic	**Eye**		
		Iris muscle	Contraction	Miosis
		Ciliary muscle		Blurred vision
		Glands		
		Lacrimal	Secretion	Tearing
		Salivary		Salivation
		Respiratory		Bronchorrhea; rhinitis; pulmonary edema
		Gastrointestinal		Nausea; vomiting; diarrhea
		Sweat		Perspiration
Sympathetic (sympatholytic)		**Heart**		
		Sinus node	Slowing	Bradycardia
		Atrioventricular (AV) node	Increased refractory period	Dysrhythmia; heart block
		Smooth Muscle		
		Bronchial	Contraction	Broncho-constriction
		Gastrointestinal		Vomiting; cramps; diarrhea
		Sphincter	Relaxation	Fecal incontinence
		Bladder		
		Fundus	Contraction	Urination
		Sphincter	Relaxation	Urinary incontinence
Neuromuscular	nicotinic	**Skeletal**	Excitation	Fasciculations; cramps followed by weakness; pupillary dilation; loss of reflexes; paralysis
		Heart	Excitation	Tachycardia
Central nervous		**Brain/Brainstem**	Excitation (early)	Headache; malaise; dizziness; confusion; manic or bizarre behavior
			Depression (late)	Depression, then loss of consciousness; respiratory depression; respiratory (diaphragm) paralysis

[a] Modified from ATSDR 1993

Table 3-2: Parameters used in DDVP air model

Parameter	Value	Units	Description/Comment/Reference
γ	37.5	Unitless	Apparent adsorption coefficient based on optimization using relative errors. See Worksheet A02b and Section 3.2.2.2 for discussion.
λ	0.023	day^{-1}	First-order release rate from Shell No-Pest Strips from Gillett et al. (1972a). Used to estimate γ from the data reported by Slomka (1970).
	0.04	day^{-1}	First-order release rate from VaporTape II strips based on data from Hercon (1994). See Worksheet A01.
RH	0.4	Unitless	Relative humidity used by Gillett et al. (1972a) and used for model application in Worksheets A02a, A02b, A03a, and A03b. This is a sensitive parameter. See text for discussion.
k	109.3	$days^{-1}$	Hydrolysis rate constant from Gillett et al. (1972a)
At*/*Va	0, 60, and 625, and 6500	day^{-1}	Air turnover rate – i.e., the ratio of air flow to room volume. Values of 0 and 60 used by Gillett et al (1972a) for no ventilation and very poor ventilation, respectively. Values of 300 and 3000 are selected as adequate ventilation for a garage and vehicle, respectively – see Section 4.4 for discussion.

Table 3-3: Summary of Risk Characterization for Human Health Risk Assessment [1]

Group	Scenario	Hazard Quotients Central	Lower	Upper	Toxicity Value	Units	Section
Workers							
	Inhalation During Assembly	**3**	0.9	**5**	0.1	mg/m^3	3.3.2.3
	Inhalation During Transport	**15**	1.0	**18**	0.1	mg/m^3	3.3.2.3
	Dermal During Assembly	0.5	0.2	**3**	0.0017	mg/kg	3.3.2.2
Child							
	Incidental Dermal Contact	**10**	**1.8**	**60**	0.0017	mg/kg	3.3.2.2
	Oral Exposure from Strip	**97**	**24**	**380**	0.0017	mg/kg	3.3.2.1
	Oral Exposure from Water	0.008	0.002	0.04	0.0017	mg/kg	3.3.2.1

[1] All of the exposure assessments on which these hazard quotients are based should be regarded as atypical and most are extreme. As noted in Section 3.2, typical exposures for workers and members of the general public will typically be negligible.

Table 4-1: Summary of Exposure Assessments and Risk Characterization for Non-target Species

Exposure Assessments						
		Estimated Exposures				
Species	**Scenario**	**Central**	**Lower**	**Upper**	**Units**	**Worksheet**
Racoon	Consumption	3.16E+01	1.05E+01	1.05E+02	mg/kg	D01 as DDVP-PVC
Small mammal	Contaminated Water	2.59E-05	8.64E-06	8.64E-05	mg/kg	D02 as free DDVP
Aquatic Species	Contaminated Water	0.000177	0.000059	0.00059	mg/L	D02

Risk Characterization						
		Risk Quotients[1]			**Toxicity Value**	
Species	**Scenario**	**Central**	**Lower**	**Upper**	**Value**	**Units**
Racoon	Consumption	0.1	0.04	0.4	240	mg/kg as DDVP-PVC
Small mammal	Contaminated Water	0.0001	0.00002	0.0002	0.5	mg/kg as free DDVP
Aquatic Species						
	Fish	0.006	0.002	0.02	0.03	mg/L NOEC as free DDVP
	Sensitive Invertebrates	**3**	0.8	**8**	0.00007	mg/L LC_{50} as free DDVP
	Tolerant Invertebrates	0.00001	0.000003	0.00003	21	mg/L LC_{50} as free DDVP

[1] Risk quotients are calculated as the exposure value, given in the upper section of the table divided by the toxicity value specified for the non-target species. This ratio is rounded to one significant digit.

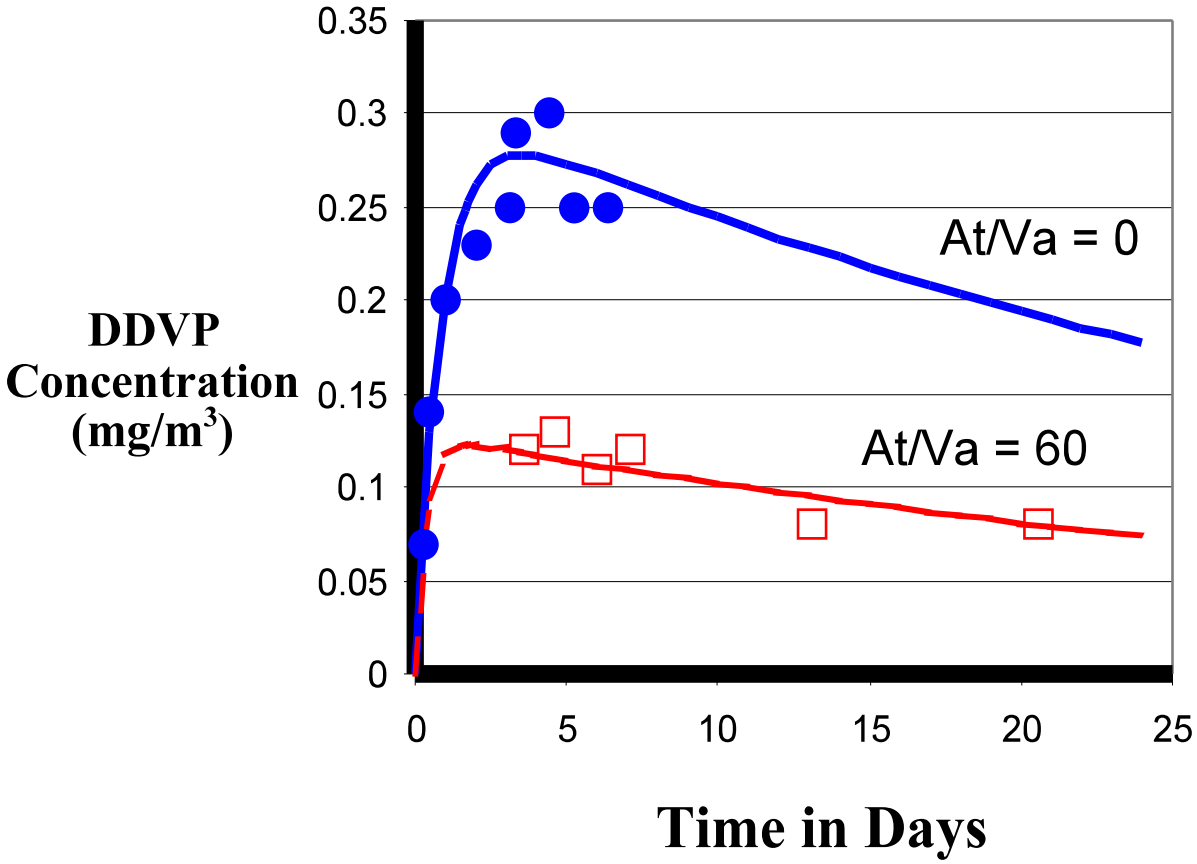

Figure 3-1: Concentration of DDVP in Air After the Placement of One Shell No-Pest Strip in an Unventilated Room (At/Va=0) and a Poorly Ventilated Room (At/Va=60)(data from Slomka 1970). See text for discussion and Worksheet A02b for details.

Appendix 1: Application and Optimization of DDVP Inhalation Exposure Model

Gillett et al. (1972a) proposed the following model for estimating concentrations of DDVP in air from the release of DDVP from PVC pest strips:

$$C_t = \frac{8}{\pi^2} \frac{M_0}{Va(1+\gamma)} \frac{\exp(-\lambda t) - \exp(\frac{-(kRH + \frac{At}{Va})}{1+\gamma} t)}{\frac{(kRH + \frac{At}{Va})}{\lambda (1+\gamma)} - 1} \qquad \text{(Eq. A-1)}$$

The terms in the above equation are defined as follows:

t	time after start of release
C_t	concentration of DDVP in air at time, *t* (days)
M_0	mass of DDVP in strip or strips at time zero (mg)
Va	volume of room or other space (m^3)
γ	apparent adsorption coefficient of DDVP on to surfaces
exp(***x***)	the exponential function, e^x, where is the constant 2.718 and ***x*** is any numeric expression
λ	first-order release rate constant ($days^{-1}$)
RH	relative humidity (proportion)
At	air flow rate (m^3/day)
k	first-order hydrolysis rate ($days^{-1}$)

and the parameters used in the model are summarized in Table 3-2.

The above equation is modified from Equation 3 in Gillett et al. (1972, p. 126). For simplicity, the term ***RH*** is used above rather than the term p/p_0 used by Gillett – i.e., the ratio of the ambient to the saturated vapor concentration of water. More significantly, the equation given in the Gillett publication – i.e., Equation 3, p. 126 – contains two typographical errors. Both errors are in the numerator to the second exponential function. The Gillett publication fails to note that the negative of the sum, ***k RH + At/Va***, must be used. These are essentially two first order processes – i.e., hydrolysis and dilution. If the negative of these values is not used, the equation models first-order growth rather than dissipation. Dissipation is clearly the intent of this term in the equation. The second more trivial error is that the ***k RH + At/Va*** term must be multiplied by ***t*** within the second exponential term. Otherwise, the units of the equation do not reduce to a concentration in air. This is analogous to the general equation for first-order absorption and first-

order elimination (e.g., Goldstein et al. 1974, p. 333). The discussion of the validation of this equation by Gillett et al. (1972a) and the implementation of this equation in the Worksheets uses the corrected form of the equation given above. Using the equation given by Gillett et al. (1972a) does not reproduce the results illustrated in Figure 4 of Gillett et al. (1972, p. 128) or in Worksheets A02a and A02b.]

Gillett et al. (1972a) applied this model to the data from Slomka (1970) in which a single Shell No-Pest Strip containing 20,000 mg of DDVP was placed rooms with a volume of 28.3 m^3 at 25°C and a relative humidity of 40%. Two different ventilation conditions were used, no ventilation and poor ventilation. No ventilation is characterized simply as a room with no air turnover – i.e., At/Va = 0. Poor ventilation is characterized as a room in which 20 air exchanges occurred per day – i.e., At/Va = 20. The apparent adsorption coefficient (γ) was treated as an empirical parameter and optimized to the data from Slomka (1970). All other model parameters were taken from the literature as specified in Table 3-2.

Gillett et al. (1972a) report an optimized value of 44.76 for the apparent adsorption coefficient (γ) but do not specify how this parameter was optimized. For the current risk assessment, the model given above was implemented in EXCEL and the data from Slomka (1970) was taken from Figure 4 in the publication of Gillett et al. (1972a). The apparent adsorption coefficient was then optimized using the EXCEL Solver function with the quasi-Newton method (with the tangent estimate and forward derivative options). Two sets of optimizations were conducted. The first was based on minimizing the standard square of error (Worksheet A02a) and the second was based on square of the relative error (Worksheet A02b). These optimizations yielded estimates of the apparent adsorption coefficient (γ) of 54.5 and 37.5, respectively, which bracket the estimate of 44.76 reported by Gillett et al. (1972a). As illustrated in Worksheets A02a and A02b, both of the optimized values fit the data from Slomka (1970) reasonably well. For the current risk assessment, the worker exposure estimates are based on the apparent adsorption coefficient (γ) 37.5, which leads to modestly higher estimates of exposure than do the higher estimates of the apparent adsorption coefficient. The fit of the Gillett et al. (1972a) model to the data from Slomka (1970) using the apparent adsorption coefficient (γ) of 37.5 is illustrated in Figure 3-1 (which is in turn taken from Worksheet A02b).

Appendix 2: Estimates of dermal absorption rates for DDVP

Table A2-1: Method for estimating the dermal permeability (K_p in cm/hr) and 95% confidence intervals.

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

[a]Mendenhall and Scheaffer, 1973, Appendix 3, Table 4, p. A31.

NOTE: The data for this analysis are taken from U.S. EPA (1992), Dermal Exposure Assessment: Principles and Applications, EPA/600/8-91/011B, Table 5-4, pp. 5-15 through 5-19. The U.S. EPA report does not provide sufficient information for the calculation of confidence intervals. The synopsis of the above analysis was conducted in STATGRAPHICS Plus for Windows, Version 3.1 (Manugistics, 1995) as well as Mathematica, Version 3.0.1.1 (Wolfram Research, 1997). Although not explicitly stated in the U.S. EPA report, 3 of the 93 data points are censored from the analysis because they are statistical outliers: [Hydrocortisone-21-yl]-hemipimelate, n-nonanol, and n-propanol. The model parameters reported above are consistent with those reported by U.S. EPA but are carried out to a greater number of decimal places to reduce rounding errors when calculating the confidence intervals. See notes to Worksheet A07a for details of calculating maximum likelihood estimates and confidence intervals.

Table A2-2**:** Calculation of dermal permeability rate (K_p) in cm/hour for DDVP.								
Parameters	Value	Units	Reference					
Molecular weight	220.98	g/mole						
$K_{o/w}$ at pH 7	29.51	unitless						
$\log_{10} K_{o/w}$	1.47							
Column vector ***a*** for calculating confidence intervals (see Worksheet A07a for definitions.)								
a_1	1							
a_2	220.98							
a_3	1.47							
Calculation of $\mathbf{a'} \cdot (\mathbf{X'X})^{-1} \cdot \mathbf{a}$ - see Worksheet A07b for details of calculation.								
Term 1	0.0190806955							
Term 2	0.001157619							
Term 3	-0.006428795							
$\mathbf{a'} \cdot (\mathbf{X'X})^{-1} \cdot \mathbf{a}$	0.0138	calculation verified in Mathematica 3.0.1.1						
$\log_{10} k_p = 0.706648 \log_{10}(k_{o/w}) - 0.006151\ MW - 2.72576$							Worksheet A07b	
$\log_{10}$ of dermal permeability								
Central estimate	-3.04623542	±	$t_{0.025}$	×	s	×	$\mathbf{a'}\cdot(\mathbf{X'X})^{-1}\cdot\mathbf{a}^{0.5}$	
Lower limit	-3.21365532088	-	1.9600	×	0.727129	×	0.1174734012	
Upper limit	-2.87881551912	+	1.9600	×	0.727129	×	0.1174734012	
Dermal permeability								
Central estimate	0.00090	cm/hour						
Lower limit	0.00061	cm/hour						
Upper limit	0.0013	cm/hour						

Details of calculating a′X′X a

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

Letting

$$\mathbf{a} = \{a\_1, a\_2, a\_3\}$$

and

$$(\mathbf{X'X})^{-1} = \{ \{b\_1, b\_2, b\_3\}, \{c\_1, c\_2, c\_3\}, \{d\_1, d\_2, d\_3\} \},$$

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

Term 1: $\{a\_1 \times([a\_1\times b\_1] + [a\_2\times c\_1] + [a\_3\times d\_1])\} +$

Term 2: $\{a\_2 \times([a\_1\times b\_2] + [a\_2\times c\_2] + [a\_3\times d\_2])\} +$

Term 3: $\{a\_3 \times([a\_1\times b\_3] + [a\_2\times c\_3] + [a\_3\times d\_3])\}.$

Appendix L
Gypsy Moth
Risk Assessment

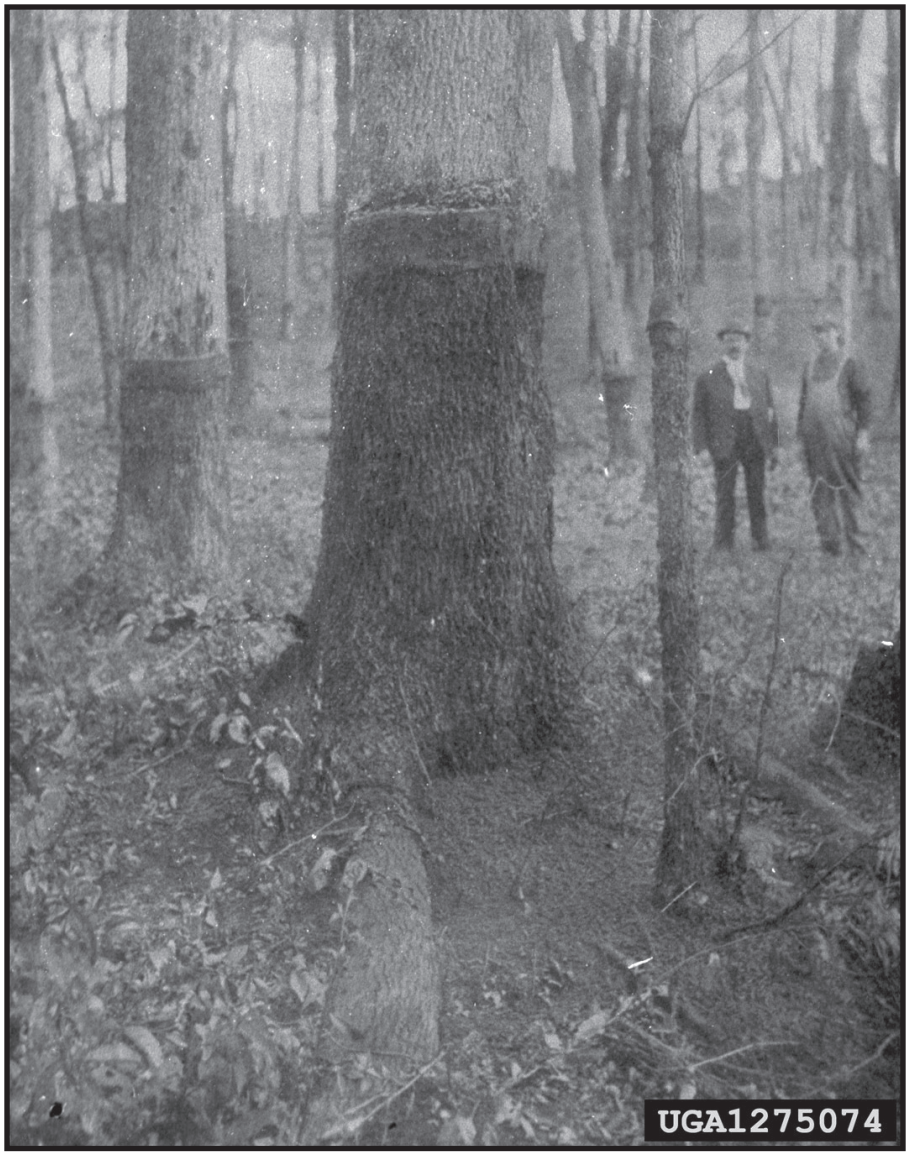

Figure L-1. Gypsy moth caterpillars cluster at the base of a banded tree (Arlington, Virginia, 1905).

Control/Eradication Agents for the Gypsy Moth - Human Health and Ecological Risk Assessment for the Gypsy Moth FINAL REPORT

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AEL	adverse-effect level
bw	body weight
B.t.k.	*Bacillus thuringiensis* var. *kurstaki*
CI	confidence interval
cm	centimeter
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
F	female
FH	Forest Health
g	gram
ha	hectare
HQ	hazard quotient
kg	kilogram
L	liter
lb	pound
LdNPV	*Lymantria dispar* (gypsy moth) nuclear polyhedrosis virus
LOAEL	lowest-observed-adverse-effect level
LOC	level of concern
m	meter
M	male
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOS	not otherwise specified
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
RfD	reference dose
SERA	Syracuse Environmental Research Associates
UF	uncertainty factor
U.S.	United States
USDA	U.S. Department of Agriculture
U.S. EPA	U.S. Environmental Protection Agency
µ	micron or micro-

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

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

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

CONVERSION OF SCIENTIFIC NOTATION

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

EXECUTIVE SUMMARY

OVERVIEW

The best documented and most obvious effect of the gypsy moth will be on terrestrial vegetation, particularly forest stands in which sensitive species of trees predominate. In sensitive forest stands, gypsy moth larvae can cause substantial defoliation. In forest stands in which tree species that are not favored by gypsy moth larvae predominate, even relatively high exposures may not result in substantial defoliation.

The gypsy moth may also have a direct impact on human health and the most likely effects will involve skin irritation. In heavy gypsy moth infestations, adverse skin reactions would be expected in substantial numbers and the effects would likely be sufficiently severe to cause some individuals to seek medical attention. In extreme outbreaks, the effects will be qualitatively similar to those of severe infestations but could affect up to about one-third of the human population.

Because the gypsy moth may substantially damage some forests in severe infestations or outbreaks, secondary effects in some species of wildlife are plausible and include reductions in populations of squirrels, mice, and other mammals which may be sensitive to changes in the availability of acorns are likely. Substantial adverse effects on other groups of animals – i.e., birds, reptiles, and aquatic species – cannot be ruled out but have not been convincingly demonstrated.

GYPSY MOTH AS A PEST SPECIES

The gypsy moth is a pest species that can cause substantial damage to some forests. In the eastern United States, most hardwood forests are classified as susceptible to gypsy moth infestation and as many as 12.5 million acres have been defoliated in a single season. The gypsy moth is found throughout much of New England and south to Virginia and west to portions of Wisconsin. The potential for substantial outbreaks is often assessed based on counts of overwintering egg masses, which are relatively easy to measure and can be made in time to plan for and take preventative measures against the outbreak.

The life cycle of the gypsy moth consists of the egg, larval, pupal, and adult stages with one generation produced each year. The larvae or caterpillars go through various sub-stages, referred to as instars. First stage larvae (first instars) hatch in early to late May and go through additional larval stages between May and late June. First instars spin fine silk threads near the tops of trees from which they suspend themselves; in the event of sufficient wind, these threads break allowing the caterpillars to be transported by the wind. The distances involved in wind dispersion may cover several miles. Between late June and mid-July, the caterpillars spin sparse silken cradles and form pupae. After 7–14 days of pupation, the adult moths emerge and mate, and the life cycle is repeated.

The gypsy moth is susceptible to diseases caused by gypsy moth pathogens like *B.t.k.*, the gypsy moth nuclear polyhedrosis virus (LdNPV), and *Entomophaga maimaiga* fungi. *B.t.k.* and LdNPV are also used as control agents for the gypsy moth and these agents are addressed individually in separate risk assessments. The gypsy moth is a prey species for some mammals, birds, and other insects. In general, invertebrates are the major predators of gypsy moth larvae, while small mammals are the major predators of pupae. Numerous insects, including the larvae of various flies and wasps, act as parasites or predators to the gypsy moth.

HUMAN HEALTH RISK ASSESSMENT

Hazard Identification – Skin irritation after contact with larvae of many species of lepidoptera is common and this effect is the most common and best documented response to contact with gypsy moth larvae. The skin reactions seem to be associated with contact with small fine hairs that stick out from the body of the larva. The precise mechanism or mechanisms of action for these irritant effects is unclear but may involve three general responses: mechanical irritation, toxic reaction to a compound such as histamine, and an immediate or delayed allergic reaction. Raised and reddened areas of skin, known as wheals, are the most characteristic skin lesions. These lesions, resembling the raised patches of skin often associated with mosquito bites, may be approximately 0.25–0.5 inches in diameter. Contact with larvae may also cause rashes rather than wheals. Both wheals and rashes may cause severe itching that can persist for several days to 2 weeks and may be sufficiently severe to cause the affected individual to seek medical treatment. Other effects that may be associated with exposure to gypsy moth larvae include eye and respiratory irritation but these effects are less well documented, compared with dermal effects.

In very severe infestations, the large numbers of larvae in an area may cause stress or anxiety in some individuals. Also during heavy infestations, water quality may be affected by increased runoff and by direct contamination with frass. Nonetheless, there are no documented cases of changes in water quality being associated with adverse effects in humans.

Exposure Assessment – The number of larvae per unit area or tree might be considered the most direct and relevant measure of human exposure because it is contact with the larvae that causes skin irritation, the adverse effect typically associated with the gypsy moth. The available dose response data, however, are based on studies in which exposure is quantified as the number of eggs masses per acre and thus this is the exposure measure that is used in this risk assessment. As long as gypsy moth populations remain sparse, the larvae usually eat only a small proportion of the foliage of even their most favored host species, and contacts with people are rare. In such cases, egg masses generally do not exceed 50 egg masses/acre. During full-scale outbreaks, densities of about 5000 egg masses/acre are common and densities greater than 20,000 egg masses/acre are occasionally recorded.

Dose-Response Assessment – The dose-response assessment for human health effects is based on reports of skin irritation in two populations: one with low exposure (an average of 32 egg masses/acre) and the other with high exposure (an average of 3809 egg masses/acre). The low-

exposure group exhibited no increase in skin irritation and 32 egg masses/acre is taken as a NOAEL (no adverse effect level) for humans and is used as a surrogate RfD (reference dose) for exposure to the gypsy moth in a manner analogous to the use of RfD values for control agents. The high exposure group did evidence a significant increase in skin irritation and, based on a dose-response model developed by U.S. EPA, egg mass densities up to 128 egg masses/acre are not likely to cause a detectable increase in skin irritation or rashes. In addition to these quantitative estimates, the severity of the response is important, particularly in a comparison of effects caused by exposure to the gypsy moth and effects caused by exposure to the agents used to control the gypsy moth. Dermal responses to the gypsy moth are sufficiently severe to have generated numerous case reports. While precise statistics are not available, it does appear that the severity of the skin irritation is sufficient to cause appreciable numbers of affected individuals to seek medical care. While exposure to the gypsy moth is associated with irritation to the eyes and respiratory tract, quantitative dose-response relationships for these endpoints cannot be developed.

Risk Characterization – In sparse to moderate infestations—i.e., egg mass densities of <500 egg masses/acre—adverse effects involving skin irritation are not likely to be detectable in populations of exposed humans. Nonetheless, some individuals who come into contact with gypsy moth larvae could develop skin irritation. In heavy gypsy moth infestations—i.e., >500 to 5000 egg masses/acre—adverse skin reactions would be expected in substantial numbers and the effects would likely be sufficiently severe to cause some individuals to seek medical attention. In extreme outbreaks—i.e., >5,000 to 20,000 egg masses/acre— the effects will be qualitatively similar to those of severe infestations but could affect up to about one-third of the population. Heavy infestations or extreme outbreaks could cause ocular and respiratory effects in some people but the likelihood of observing these effects cannot be quantified. Similarly, severe infestations are often considered to be a nuisance and cause aesthetic damage to the environment. Both of these factors can lead to stress in some individuals. Young children may be a group at special risk from effects of gypsy moth exposure but it is not clear whether children are more sensitive than adults to the effects of gypsy moth exposure or whether responses in children appear greater because children spend more time outdoors compared with adults.

ECOLOGICAL RISK ASSESSMENT

Hazard Identification – The clearest primary effect of gypsy moth infestations is on terrestrial plants, primarily trees. Various instars of the gypsy moth larvae will feed on host trees and can cause extensive defoliation which can kill some of the infested trees. On a larger scale, the extensive defoliation and/or death of trees may result in secondary changes to vegetation, which will, in turn, affect other forms of vegetation as well as various animal species (primarily related to changes in habitat). Gypsy moth larvae appear to have definite food preferences; oak, birch, poplar, and apple trees seem to be their favorite food sources. While both the European and Asian gypsy moth cause similar types of damage (i.e., defoliation), their feeding preferences are somewhat different with the Asian gypsy moth preferring a wider range of vegetation. Heavy defoliation is much more common among the oaks than among trees that are not particularly favored as food by the gypsy moth. For susceptible oaks, the effects of infestations on tree

mortality varies according to the initial condition of the stand and the number of infestations. Generally, gypsy moth infestations result in mortality of less than 15% of total basal area – i.e., mortality of trees involving 15% the total area of the tree trunks near the ground. When heavy defoliation is followed by massive overstory mortality, existing shrub and herb cover increase dramatically due to increases in available light, moisture, and nutrients. Extensive loss of the existing canopy will also favor the growth of tree species that are intolerant to shade and will shift the forest ecosystem towards earlier successional stages.

The only other groups of organisms that are likely to be directly affected by the gypsy moth are some and probably very few other lepidopteran species, including the northern tiger swallowtail butterfly. The mechanisms for direct adverse effects on other lepidopteran species may include bacterial contamination of the leaves by gypsy moth larvae and a decrease in the nutritional value of the leaves damaged by the gypsy moth. Most studies, however, do not indicate substantial direct effects on other insects, including lepidoptera. In some cases, increases may be seen in populations of insect predators of the gypsy moth.

There is no indication in the literature that the gypsy moth will cause direct adverse effects in most groups of animals. Indirect effects, associated with damage to vegetation, may be of substantial consequence to some species, including squirrels, mice, and other mammals that rely on acorns. Although some mammals consume insects, including the gypsy moth, there is no evidence that gypsy moth outbreaks have a substantial impact on insectivorous mammals. Similarly, there is little indication that birds or aquatic species will be adversely affected by the gypsy moth. In some species of birds, gypsy moth infestations and subsequent defoliation may be beneficial, especially for species of birds that favor dead wood as a habitat.

Exposure Assessment – As in the human health risk assessment, the exposure metameter is dictated by the data used to formulate the dose-response assessment. Also as in the human health risk assessment, egg mass density is the exposure metameter for terrestrial invertebrates and plants because it is the measure on which the dose-response assessment is based. Egg mass densities spanning a range from 5 egg masses/acre to 5,000 egg masses per acre are used to estimate responses in terrestrial plants and invertebrates.

Most wildlife species are not affected directly by exposure to the gypsy moth but are more likely to experience indirect effects like changes in habitat or other environmental conditions secondary to defoliation. Consequently, the exposure assessment for most wildlife species is almost identical to the dose-response assessment for terrestrial plants which is expressed as defoliation caused by gypsy moth larvae. For this exposure assessment, categories of defoliation are defined normal background defoliation (<30% defoliation), moderate defoliation (30-60% defoliation), and high or severe defoliation (>60% defoliation).

Dose-Response Assessment – As in the human health risk assessment, the dose metameter is egg masses/acre. Quantitative dose-response assessments can be made for both terrestrial plants and sensitive species of lepidoptera. The dose-response assessments for terrestrial plants are based

on a relatively simple quantitative model for the relationship of egg mass density to defoliation. Three broad categories (sensitive, intermediate, and tolerant) are used to characterize the susceptibility of forest stands to gypsy moth induced defoliation. Estimated LOAEL values based on 30% defoliation, which is considered the lower range of moderate defoliation, are approximately 125, 1000, and 7000 egg masses/acre for sensitive, intermediate, and tolerant forest stands, respectively. The corresponding NOAEL values, defined as 10% defoliation, are estimated as 12, 20, and 125 egg masses/acre for sensitive, intermediate, and tolerant forest stands.

The effects of gypsy moth exposure on sensitive terrestrial invertebrates, including some species of lepidoptera, are less well documented and less well characterized, compared with the effects on terrestrial plants. Nonetheless, available studies indicate that the NOAEL for adverse effects in certain other species of lepidoptera are lower than the NOAEL for sensitive forest stands—— i.e., about 6-72 egg masses/acre for some lepidoptera.

No quantitative dose-response assessment is presented for other groups of organisms—e.g., mammals, birds, and soil or aquatic organisms. The impact of gypsy moth exposure on these species is most likely to result in indirect effects secondary to defoliation.

Risk Characterization – The best documented and most obvious effect of the gypsy moth will be on terrestrial vegetation, particularly forest stands in which sensitive species of trees predominate. In some respects, the risk characterization for terrestrial vegetation is essentially a restatement of the hazard identification. In other words, the effects of gypsy moth larvae on forests is extremely well documented and relatively well understood. In sensitive forest stands—i.e., stands in which oak, birch, and other favored species predominate —gypsy moth larvae can cause substantial defoliation. In forest stands in which tree species that are not favored by gypsy moth larvae predominate —e.g., hemlock, various types of pine, black locust and white ash—even relatively high exposures, as measured by egg mass density, may not result in substantial defoliation. The risk assessment for direct effects on forests should be at least qualitatively influenced by the current range of the gypsy moth, which has not yet extended to some forests in the southeast that may be among the most sensitive to gypsy moth exposure. Thus, unless measures to contain the gypsy moth are successful, the southeastern oak forests may suffer serious damage in future infestations.

Some other lepidopteran species also may be directly affected by exposure to the gypsy moth. Most studies, however, suggest that substantial adverse effects in terrestrial insects are unlikely and effects in some insect species, including some other lepidoptera, may be beneficial.

Because the gypsy moth may substantially damage some forests in severe infestations or outbreaks, secondary effects in other species of wildlife are plausible. Reductions in populations of squirrels, mice, and other mammals which may be sensitive to changes in the availability of acorns are likely. Substantial adverse effects on other groups of animals – i.e., birds, reptiles, and aquatic species – cannot be ruled out but have not been convincingly demonstrated.

1. INTRODUCTION

This report addresses the potential human health effects and ecological effects of gypsy moth infestations and is part of the effort to update the 1995 Final Environmental Impact Statement (FEIS) for the Cooperative Gypsy Moth Management Program. The effort to update the FEIS involves the preparation of human health risk assessments (HHRAs) and ecological risk assessments (ERAs) for each of the agents used to control or eradicate gypsy moth infestations: *Bacillus thuringiensis kurstaki* (*B.t.k.*), Gypchek, diflubenzuron, tebufenozide, DDVP and disparlure. This risk assessment of the gypsy moth is intended to assist the USDA in assessing the consequences of "no action" alternatives in the FEIS. In addition, a separate document in this series will compare the effects gypsy moth infestations with the effects of the agents used to control the infestations.

This documents consists of an introduction, an overview of the gypsy moth as a pest species (Section 2), a risk assessment for human health effects (Section 3), and a risk assessment for ecological effects or effects on non-target wildlife species (Section 4). Each of the two risk assessment chapters has four major sections, including an identification of the hazards associated with the gypsy moth, an assessment of potential exposure to the gypsy moth, an assessment of the dose-response relationships, and a characterization of the risks associated with plausible levels of exposure. These are the basic steps recommended by the National Research Council of the National Academy of Sciences (NRC 1983) for conducting and organizing risk assessments.

The risk assessment on the gypsy moth is different from the risk assessments for chemical and biological agents used to control gypsy moth infestations, primarily because many standard physical and chemical properties used to characterize control agents and estimate certain exposure parameters are not at issue. Moreover, estimates of human and ecological exposure to all control agents—chemical and biological— are based on application rates, (i.e., known amounts of the agent applied under reasonably well defined conditions), which are not relevant to the gypsy moth. As discussed in subsequent sections of this document, estimates regarding gypsy moth exposure are extremely variable and difficult to define.

A tremendous body of information is available on the biology, physiology, and population dynamics of the gypsy moth and this information is presented in reviews, books, and monographs that are available in the open literature (e.g., Davidson et al. 1999, 2001; Gansner et al. 1993a; Gerardi and Grimm 1979; Herrick and Gansner 1988; Liebhold 1992; Nealis et al. 1999; Sharov et al. 1999, 2002; Wallner 1994, 1996; Williams et al. 2000). Additional information on the gypsy moth is available at a USDA Forest Service web site, http://na.fs.fed.us/wv/gmdigest/. The current risk assessment makes no attempt to summarize all of this information. Although some background information is presented (Section 2), the primary focus of this document is on the information that can be used directly to assess the human health effects (Section 3) and ecological effects (Section 4) of the gypsy moth in ways that correspond to and may be compared to the risk assessments of agents used to control or eradicate gypsy moth infestations.

This is a technical support document that addresses some specialized technical areas. Nevertheless, an effort was made to ensure that the document can be understood by individuals who do not have specialized training in the chemical and biological sciences. Certain technical concepts, methods, and terms common to most risk assessments are described in a separate document (SERA 2001). In addition, general glossaries of environmental terms are widely available and a custom glossary designed to be used in conjunction with USDA risk assessments is available at www.sera-inc.com. Some of the more complicated terms that are specific to the gypsy moth are defined in the text of this risk assessment.

2. GYPSY MOTH AS PEST SPECIES

2.1. OVERVIEW

The gypsy moth is a pest species that can cause substantial damage to some forests. In the eastern United States, most hardwood forests are classified as susceptible to gypsy moth infestation and as many as 12.5 million acres have been defoliated in a single season. The gypsy moth is found throughout much of New England and south to Virginia and west to portions of Wisconsin. The potential for substantial outbreaks is often assessed based on counts of overwintering egg masses, which are relatively easy to measure and can be made in time to plan for and take preventative measures against the outbreak.

The life cycle of the gypsy moth consists of the egg, larval, pupal, and adult stages with one generation produced each year. The larvae or caterpillars go through various sub-stages, referred to as instars. First stage larvae (first instars) hatch in early to late May and go through additional larval stages between May and late June. First instars spin fine silk threads near the tops of trees from which they suspend themselves; in the event of sufficient wind, these threads break allowing the caterpillars to be transported for long distances by the wind. Between late June and mid-July, the caterpillars spin sparse silken cradles and form pupae. After 7–14 days of pupation, the adult moths emerge and mate, and the life cycle is repeated.

The gypsy moth is susceptible to diseases caused by gypsy moth pathogens including *B.t.k.*, the gypsy moth nuclear polyhedrosis virus (LdNPV), and *Entomophaga maimaiga* fungi. *B.t.k.* and LdNPV are also used as control agents for the gypsy moth and these agents are addressed individually in separate risk assessments. The gypsy moth is a prey species for some mammals, birds, and other insects. In general, invertebrates are the major predators of gypsy moth larvae, while small mammals are the major predators of pupae. Numerous insects, including the larvae of various flies and wasps, act as parasites or predators to the gypsy moth.

2.2. INFESTATIONS

The current scientific name is for the gypsy moth is *Lymantria dispar*. In the older literature (e.g., Gerardi and Grimm 1979), the gypsy moth is referred to by its previous scientific name, *Porthetria dispar*. Over three quarters of the hardwood forests in the eastern United States are classified as susceptible to the gypsy moth (USDA/FS 1990). In addition, many forests in the south and central regions of the country, currently beyond the range of the gypsy moth, are likely to be very susceptible to damage by the gypsy moth (Liebhold and McManus 1999). In a major outbreak, the extent of damage can be substantial and as many as 12.5 million acres have been defoliated in a single season (Williams 1982). Damage to vegetation is caused by feeding larvae. During outbreaks, gypsy moth larval populations may range from about 10,000 to 250,000 larvae per hectare (Colbert et al. 1995; Christie et al. 1995).

The gypsy moth was brought into the United States intentionally in 1869 as part of an experiment by a naturalist, Leopald Trouvelet, to develop a hardy silk-producing insect. In the course of the experiments, conducted in Medford, Massachusetts, some gypsy moth eggs were lost and a

population of gypsy moths was established in the Medford area. The gypsy moth population grew to infest about a 400 square mile area around Medford by 1880, and the first major outbreak occurred in 1889 (Gerardi and Grimm 1979). The gypsy moth has spread throughout much of New England and south to Virginia and east to portions of Wisconsin. Current and plausible future infestations are discussed further in the exposure assessment for human health effects (Section 3.2) and ecological effects (Section 4.2). Figure 2-1 summarizes information regarding the frequency of gypsy moth defoliations over a period of 28 years—i.e., 1975 to 2002. In any given year, marked defoliations associated with gypsy moth infestations may be less ubiquitous and may be isolated in relatively small areas, which is due both to the control measures taken to limit gypsy moth populations as well as to the natural variability in gypsy moth populations.

The population pattern observed after the release of the first gypsy moths in North America—i.e., a period of low and inconsequential population growth followed by a major outbreak—is typical of gypsy moth population dynamics, which are described as bimodal (i.e., existing either at innocuous densities or in an outbreak or very rapid growth mode) (Campbell 1981). Following an initial outbreak, populations generally decline and are usually maintained at low population densities that cause little damage. Subsequent outbreaks are usually less severe than the initial outbreak. As discussed further in Section 4.2, gypsy moth outbreaks are often associated with the presence of favored tree species (Baker and Cline 1936; Behre 1939; Behre and Reineke 1943). In general, gypsy moth outbreaks in North America dissipate in 1or 2 years. In rare cases, outbreaks can recur annually over periods of up to 20 years (Bess et al. 1947; Campbell 1973).

For at least half a century, the gypsy moth has persisted at generally innocuous densities in the predominantly oak forests of northeastern Connecticut and adjacent Massachusetts (Bess et al. 1947; Brown and Sheals 1944). During such intervals, gypsy moth larvae usually eat only a small proportion of the foliage of even their most favored host species. When defoliation is low, nearly all of it occurs on favored-food trees (Campbell and Sloan 1977b). Once a large-scale outbreak is underway, the gypsy moth will feed on a greater variety of vegetation and over 300 species of broadleaf and coniferous trees and shrubs may be damaged (Leonard 1981; Liebhold et al. 1994).

The potential for substantial outbreaks is often assessed based on counts of overwintering egg masses. Such counts are relatively easy to make and can be made in time to plan for and take preventative measures against a potential outbreak (Buss et al. 1999). Egg mass counts, however, are not absolute predictors of outbreak potential and egg masses per acre can be highly variable. In an infested area in Maryland, egg masses ranged from about 20/ha to 14,000/ha at 16 sites over a 4-year period (Davidson et al. 2001). In heavily infested areas, eggs masses per acre can range from about 5000 to 43,000 (Hajek 1997). The relationship of egg mass density to subsequent damage is complicated by the fact that the survival of egg masses is also highly variable, ranging from <1% to about 90% (Nealis et al. 1999). In areas with extremely cold winters, egg masses laid below the snow line tend to have higher survival rates than those laid above the snow line (Nealis et al. 1999; Smitley et al. 1998). Bess (1961) reports that reduced

defoliation, which followed a winter of prolonged below zero temperatures, was due to 90% overwintering egg mortality.

The spread of an infestation may also be influenced by available vegetation. While some studies show that the quality of resources available to the female gypsy moth has only a minor effect on population dynamics (Erelli and Elkinton 2000), other sources indicate that the consumption of vegetation by larvae and subsequent larval growth may differ substantially according to vegetation type (Foss and Rieske 2003).

The spatial distribution of stand susceptibility is a key characteristic in the spread of outbreaks and subsequent defoliation (Liebhold and McManus 1991). Outbreaks are described as originating in small, discrete locations. These locations, referred to as foci, are usually characterized by stands growing on stressed sites like ridge tops, upper slopes, and deep sands, frequently subject to drought (Houston and Valentine 1977). These areas can support moderate to high populations of gypsy moth when the insect is undetectable in surrounding areas (Liebhold and McManus 1991). Protected resting locations that favor larval and pupal survival are known to support larger gypsy moth populations and lead to outbreaks (Bess et al. 1947; Campbell and Sloan 1977a; Houston 1975; Houston and Valentine 1977).

Other factors that may precipitate outbreaks include predator failure and specific climatic and meteorological conditions. Khanislamov and Girfanova (1964) demonstrate that weather variation may have more drastic effects on the natural enemies of gypsy moth than on the pest itself. Population collapse at the end of an outbreak appears to be the result of disease (Section 2.4), reduced fecundity, and starvation (Campbell 1981). Although dispersal of young larvae plays a role in gypsy moth outbreaks, it is thought to play a relatively minor role in outbreak initiation. Larval dispersion may be the major cause of gypsy moth distribution enlargement and range expansion at innocuous densities but it does not appear to cause outbreaks to spread (Campbell 1976). The rate at which infestations spread may vary substantially according to vegetation type and the methods used to control the spread. Reported rates of infestation range from 12 to 145 km/year (Sharov et al. 1999; Wallner 1996).

There are various models to predict the effects of gypsy moth infestations on a mixed hardwood forest (Colbert and Racin 1995; Colbert et al. 1995; Weseloh 1996a,b; Wilder et al. 1995; Williams et al. 1997). These models are discussed further in the dose-response assessment for terrestrial vegetation (Section 4.3.2).

2.3. LIFE-CYCLE

As with most insects, the life cycle of the gypsy moth consists of the egg, larval, pupal, and adult stages (Abrahamson and Klass 1982; Cram 1990; Gerardi and Grimm 1979). In the northeast, the adult female lays eggs in July or August. The larvae or caterpillars go through various stages, referred to as instars. First stage larvae (first instars) hatch in early to late May and go through additional larval stages (a total of five instars in males and six instars in females) between May and late June. This process occurs somewhat earlier in the southeast. The transition from each

stage to the next involves molting, during which time the caterpillar sheds its outer skin. It is during the larval stages that feeding occurs. First instars spin fine silk threads near the tops of trees from which they suspend themselves. After the thread breaks, the larvae can be transported over relatively long distances by the wind. The distances that larvae might be carried by wind is likely to be highly variable and has not been well or generally characterized. Gerardi and Grimm (1979, p. 63) note that larvae have been monitored at elevations of up to 2000 feet and have been found at distances of up to 35 miles from the closest known infestation. Between late June and mid-July, the caterpillars spin sparse silken cradles and form pupae. After 7–14 days of pupation, the adult moths emerge and mate, and the life cycle is repeated.

Newly hatched larvae often remain on the egg mass for several days before climbing toward foliage. First-instar gypsy moth larvae have two types of hairs or setae: long thin hairs that appear to assist the larvae in "soaring" or transport by wind and short hairs (sometimes referred to as "balloon hair") that contain chemicals like nicotine which may serve as a defense mechanism to discourage predators (Bardwell and Averill 1996; Deml and Dettner 1995; Smith 1985).

Moths begin to emerge about the middle of July, with males appearing several days earlier than females. In the south, moth emergence may occur as early as June. The European female cannot fly; she emits a pheromone (sex attractant) that volatilizes and is carried in the air. Male moths are attracted to the pheromone for distances up to 1 mile. After fertilizing and depositing eggs the adult moths do not eat and soon die (Johnson and Lyon 1988). Egg masses are deposited on tree trunks, rocks, and litter. Although the eggs overwinter, below normal temperatures can cause egg mortality (Bess 1961).

This risk assessment considers both the European and Asian gypsy moths, which are considered to be the same species (*Lymantria dispar*). Since the European gypsy moth was introduced in North America from closely related individuals, genetic studies indicate little variation within or between populations. The Asian gypsy moth, on the other hand, displays considerable variability within populations. The variability is expressed morphologically in the variety of larval color forms, behaviorally in the female flight capability, and physiologically in the capacity of larvae to colonize aggressively a broad spectrum of hosts (USDA/FS 1992).

While the female European gypsy moth is flightless, the Asian female is a strong flier capable of flights in excess of 18 miles (30 km) (USDA/FS 1992; Wallner 1996). Since the female Asian gypsy moth is able to lay eggs far from the pupal site following flight, this characteristic alone may make it necessary to modify the control methods of detection, delimitation, and control or eradication developed for the European gypsy moth (USDA/FS 1992). Asian gypsy moth larvae tend to feed more aggressively and on a broader variety of trees than their European counterpart (Wallner 1996).

2.4. DISEASE AND PREDATION

The primary focus of this risk assessment is effects of the gypsy moth on other species. Nonetheless, many organisms may adversely affect the gypsy moth, thereby reducing the risks posed by gypsy moth infestations. The gypsy moth is susceptible to diseases, including diseases caused by pathogens like *B.t.k.*, the gypsy moth nuclear polyhedrosis virus (LdNPV), and *Entomophaga maimaiga* fungi. Bacterial pathogens in addition to *B.t.k.* and other *Bacillus* species that adversely affect the gypsy moth, include *Serratia marcescens, Serratia liquefaciens, Streptococcus,* and *Pseudomonas* spp. These microorganisms are associated with a collective mortality in the gypsy moth of less than or equal to 15% (Podgwaite 1981). The gypsy moth nucleopolyhedrosis virus (LdNPV) is a natural component of the gypsy moth environment (Podgwaite 1979; 1981; Podgwaite and Campbell 1970; Lindroth et al. 1999) and is considered the primary natural regulator of dense gypsy moth populations in North American forests (Glaser and Chapman 1913; Doane 1970). High density populations of gypsy moth will eventually collapse, for the most part due to pathogens, especially NPV (Elkinton and Liebhold 1990). *B.t.k.* and LdNPV are also control agents for the gypsy moth, and are addressed individually in separate risk assessments.

In addition to viral and bacterial pathogens, several fungal pathogens will infect gypsy moth populations, including species of *Paecilomyces*, *Fusarium* and *Verticillium* (Hajek 1997). Most fungal pathogens, however, appear to account for insignificant levels of recorded gypsy moth mortality (Podgwaite 1981). A major exception, however, is *Entomophaga maimaiga*, which plays an important role in gypsy moth population dynamics on other continents and which is widely established in North America. Nealis et al. (1999) estimate that *E. maimaiga* may account for approximately 4-14% of mortality in gypsy moth larvae. Infections with *E. maimaiga* tend to be more prevalent than naturally occurring infections from NPV in areas with low egg mass density (Buss et al. 1999). In low density plots, *E. maimaiga* increased mortality substantially only in 5th instar and later instars. In high density plots, earlier instars were also infected (Hajek 1997; Hajek et al. 2001). Models for the influence of *E. maimaiga* on gypsy moth populations have been developed by Weseloh (1998a, 1999, 2002, 2003).

The gypsy moth is at risk of significant predation by mammals, birds, and other insects. In general, invertebrates are the major predators of gypsy moth larvae, while small mammals are the major predators of pupae (Grushecky et al. 1998). Mice and shrews are important predators of gypsy moth, particularly during the pupal stage (Bess et al 1947; Jones et al. 1998) or when the population density of the gypsy moth is low (Elkinton et al. 1996, 2002). When the population density of small mammals is high, small mammals may be a major source of predation on larvae (Cook et al. 1995). When populations of small mammals are low, the relative importance of predation by terrestrial invertebrates increases (Hastings et al. 2002a,b).

Forbush and Fernald (1896) first identified birds as predators of gypsy moth larvae. Some species of birds even prey on egg masses (Cooper and Smith 1995). In general, however, mammals appear to have a greater impact on gypsy moth populations than birds (Smith and Lautenschlager 1981; Elkinton and Liebhold 1990).

Numerous insects act as parasites or predators to the gypsy moth, including the larvae of various tachinid flies and braconid wasps (Hajek 1997). Extensive efforts were made to introduce European and Asian gypsy moth parasitoids to North America (parasitoids are insects, especially flies and wasps, that complete their larval development inside the body of another insect). Ten species have become established (Elkinton and Liebhold 1990). Gypsy moth mortality due to each type of parasite is specific to a given gypsy moth life stage. The venom of the ectoparasitic wasp *Microbracon hebetor*, contains a toxin that inhibits larval growth in gypsy moth (Masler and Kovaleva 1999). The food preference of certain wasps species—i.e., chalcids—seems to depend on the sex of the pupae (Fuester and Taylor 1996). Although invertebrate predation of gypsy moth pupae may be minor compared with vertebrate predation (Campbell and Sloan 1977a), Smith and Lautenschlager (1981) suggest that mortality attributed to vertebrates may be caused by invertebrates, like ground beetles (Elkinton and Liebhold 1990). Both adult and immature stages of *Calosoma sycophanta*, a large ground beetle introduced from Europe, are known to feed on gypsy moth larvae and pupae (Elkinton and Liebhold 1990). In addition, Weseloh (1996b, 1998b) suggest that predation by ants, particularly on gypsy moth larvae that fall to the forest floor, could cause significant mortality to gypsy moth larvae.

3. HUMAN RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION

3.1.1. Overview

Skin irritation after contact with larvae of many species of lepidoptera is common and this effect is the most common and best documented response to contact with gypsy moth larvae. The skin reactions seem to be associated with contact with small fine hairs that stick out from the body of the larva. The precise mechanism or mechanisms of action for these irritant effects is unclear but may involve three general responses: mechanical irritation, toxic reaction to a compound such as histamine, and an immediate or delayed allergic reaction. Raised and reddened areas of skin, known as wheals, are the most characteristic skin lesions. These lesions, resembling the raised patches of skin often associated with mosquito bites, may be approximately 0.25–0.5 inches in diameter. Contact with larvae may also cause rashes rather than wheals. Both wheals and rashes may cause severe itching that can persist for several days to 2 weeks and may be sufficiently severe to cause the affected individual to seek medical treatment. Other effects that may be associated with exposure to gypsy moth larvae include eye and respiratory irritation but these effects are less well documented, compared with dermal effects.

In very severe infestations, the large numbers of larvae in an area may cause stress or anxiety in some individuals. Also during heavy infestations, water quality may be affected by increased runoff and by direct contamination with frass. Nonetheless, there are no documented cases of changes in water quality being associated with adverse effects in humans.

3.1.2. Mechanisms of Action

As discussed in Section 3.1.3, dermal irritation is the most common adverse effect associated with human exposure to gypsy moth larvae. Dermal reactions to contact with lepidopteran larvae are in general relatively common (Anonymous 1984; Gilmer 1925; Goldman et al. 1960; Hellier and Warin 1967; Katzenellenbogen 1955; Perlman 1965; Schmidt 1982; Wirtz 1980,1984). Moreover, the gypsy moth is the most common insect associated with allergies—i.e., 28.7% of known cases (Wirtz 1980).

The skin reactions seem to be associated with contact with the larval setae, small fine hair-like protrusions from the body of the larvae (Allen et al. 1991). The precise mechanism or mechanisms of action for these irritant effects is unclear but may involve three general responses: mechanical irritation, toxic reaction to a compound such as histamine, and an immediate or delayed allergic reaction (Burnett et al. 1989, Shama et al. 1982). Gypsy moth larvae have four kinds of setae, two of which are hollow and attached to glandular cells. The hollow setae are suspect, but not unequivocally identified as the setae associated with skin reactions in humans (Anderson and Furniss 1983). According to several case reports and epidemiology studies, dermal effects in humans are usually associated with exposure to the first instars (Anderson and Furniss;1983, Tuthill et al. 1984). Whole first instars and the setae of fifth instars contain histamine (Shama et al. 1982), a compound that causes wheals, which are characteristic of dermal contact with gypsy moth larvae (Sullivan 1982).

The study by Beaucher and Farnham (1982) supports the association between gypsy moth exposure and allergic responses. In the study, closed patch tests were conducted on 8 individuals who had a history of skin reactions to the gypsy moth and 11 individuals, with no such history, who served as controls. A positive response to the patch test was observed in each of the individuals who had a history of skin reactions to the gypsy moth and in only one individual in the control group. The observed response was consistent with the reported dermal effects of gypsy moth exposure. In some cases, severe itching (pruritis) kept individuals awake at night. In general, the time from exposure to the onset of the reaction was 24–48 hours, suggesting a delayed hypersensitivity similar to poison ivy reactions. In another study, 10 of 17 workers at a laboratory conducting research on the gypsy moth reported a history of adverse skin or respiratory reactions. According to the results of scratch tests, 7 of the 10 workers who reported a history of adverse reactions were allergic to gypsy moth parts or other gypsy moth substances. The intensity of the response, based on a categorical classification of skin responses, was greater for extracts of cast larval skins and whole larvae than for egg mass hairs (Etkind et al. 1982).

3.1.3. Effects on Skin

Reports of dermal responses to contact with gypsy moth larvae began with the introduction of the moth to the United States. A late 19th century document describes a situation in which an individual in Medford Massachusetts *"... was poisoned by them [gypsy moths]. While killing them upon the trees they would get upon his neck and blister and poison it"* (Forbush and Fernald 1896, p. 16). A few years later, a physician in Boston reported a number of cases of *"...inflammation of the skin, which were undoubtedly caused by contact with some caterpillar ... which must be some recently introduced species"* (White 1901 p. 599). Although Dr. White attributed these cases to the brown-tailed moth (*Euproctis chrysorrhea*), they are consistent with the reported effects of exposure to the gypsy moth which had escaped into the area near Boston some years before (Section 2.2). The literature contains no further mention of human health effects associated with the gypsy moth for almost a century.

In the early 1980s, there was a massive gypsy moth infestation in the northeastern part of the United States. In 1981, outbreaks of itchy skin rashes that coincided with the heavy infestations were widespread and a source of public annoyance (Marshall 1981). Coincident with this infestation, reports describing the human health effects associated with exposure to the gypsy moth appeared in the medical literature.

Wheals, raised and reddened areas of skin, are the most characteristic skin lesions associated with human contact with the larvae. These lesions, resembling the raised patches of skin often associated with mosquito bites, may be approximately 0.25–0.5 inches in diameter and are surrounded by an area of redness. In severe cases, the wheals may be so numerous that they overlap on large areas of the skin, a condition referred to as urticaria. Contact with the larvae is reported also to cause contact dermatitis, characterized by a rash rather than wheals (Anderson and Furniss 1983). Both rashes and wheals may cause severe itching, also known as pruritis. This effect can persist for several days to 2 weeks and may be sufficiently severe to cause the the

affected individual to seek medical treatment (Aber et al. 1982; Allen et al. 1991; Shama et al. 1982).

During the severe infestations in the early 1980s, there were three published reports regarding the development of skin reactions in school children (Aber et al. 1982; Anderson and Furniss 1983; Tuthill et al. 1984). In the spring of 1982, a telephone survey was conducted to collect information from approximately 1000 people (representing more than 90% of those selected for study) in one highly infested community (HI, Lunenburg) and one minimally infested community (LO, Medway) in Massachusetts (Tuthill et al. 1984). The risk of developing a dermal response over a 1-week period was 10.4% in the HI community and 1.6% in the LO community. The responses occurred most often in individuals who had developed rashes during the previous year or who had direct contact with the larvae (that is, larvae crawled on them). The combination of these two factors resulted in an additive increase in risk. Other variables related to increased response included a history of hay fever and the practice of hanging clothes outdoors to dry. The rates at which the dermal responses developed in individuals in the HI community were inversely associated with age (18.8% in 0- to 12-year olds, 10.2% in 13- to 59-year olds, and 2.1% in 60-year olds and older individuals). The average prevalence of dermal responses in both communities combined, 1 week before the emergence of the first instars, was 1.3% (Tuthill et al. 1984).

Sometime between the end of April and the third week of May, 1981, there was an increased incidence of rashes among students in two schools in Northeast Pennsylvania (Aber et al. 1982). School A had a response rate of 42.2% (135 of 320 students), and school B had a response rate of 25.3% (76 of 300 students). The dermal responses included pruritic rash and occasional urticaria, usually located on exposed areas of the body. Based on the results of a survey of students from the same schools who were not affected by the gypsy moth, the investigators determined that there was a statistical association between touching larvae ($p<0.01$), working in a garden ($p<0.05$), or going fishing ($p<0.01$) and the incidence of rashes.

Concurrent with the infestation in Pennsylvania was an infestation in Connecticut, associated with an outbreak of skin reactions in students at several schools within the community (Anderson and Furniss 1983). Urticaria was observed in 7.2% of the 2600 students attending four schools in Newton, Connecticut. More than 50% of the cases of urticaria occurred during the first week in May, coinciding with the emergence of first instars. Very few cases (approximately 10) occurred during the third week of May when the larvae were predominantly in the third instar stage. In Burlington, Connecticut, the incidence of skin reactions was approximately 5.1% (96 of 1870). In another school, about 7.1% (75 of 1058) of students were affected. In Bristol, Connecticut, there were 1348 cases of rashes in the public schools, amounting to approximately 10.7% of the total student enrollment (12,500). Health officials estimated that the true prevalence may have been 3 times higher than reported; however, details supporting this assessment were not provided. Nonetheless, the estimate is consistent with the occurrence of rashes in 12 of 25 children attending a nursery school in the same community.

3.1.4. Effects on Eyes and Respiratory Tract

The ocular and respiratory effects in humans after exposure to the gypsy moth or other lepidopteran larvae are less well documented, compared with dermal effects. Of the 10 workers with a history of adverse reactions to the gypsy moth (Etkind et al. 1982), all 10 had skin reactions, 4 had eye irritation, and 2 had respiratory reactions. In a survey of laboratories conducting research on insects, 28.7% of all reported allergies were attributed to the gypsy moth. The most frequent reactions among affected individuals were skin irritation (61%), sneezing or runny nose (67%), and eye irritation (60.9%). Labored respiration was observed in 33% of the affected individuals (Wirtz 1980). The frequencies of these reactions are for all individuals who had adverse health effects after exposure to insects in general, not just the gypsy moth. In the early 1980s, NIOSH conducted a survey of workers in USDA/ARS research facilities who were involved in rearing insects for various research projects. As in the study by Wirtz (1980), the most common respiratory or ocular symptoms included sneezing/runny nose (73%), ocular irritation (68%), cough (38%), wheezing (26%), and shortness of breath (24%) (Anonymous 1984). An update of this survey was planned for the early 1990 (Petsonk 1994) but no such publication was found in the literature.

The severity of ocular or respiratory effects in humans after exposure to the gypsy moth is not well characterized; however, these effects appear to be reversible. Although some respiratory effects may involve pain, there are no data to indicate that the respiratory effects are life threatening or require hospitalization (Perlman 1965; Shama et al. 1982).

3.1.5. Other Potential Effects

The stress or anxiety associated with gypsy moth infestations is difficult to assess. This stress has not been associated with frank health effects. In many communities, the stress may be exacerbated by disputes about appropriate approaches for dealing with the pest (Williams 1982). Anecdotal reports suggest that some people may be extremely anxious about infestations (National Gypsy Moth Management Group 1991, p. 3):

> *... the mere mention of insects sends some people into fits of scratching, but phobia was not an adequate explanation for the epidemic of runny noses, irritated eyes, and rashes that happened to coincide with the occurrence of gypsy moth caterpillars last spring [1990]. Every [Pennsylvania] county and state gypsy moth office received numerous calls and one agency was reported to have received over 2,700.*

Moreover, reports regarding the willingness of populations to pay for gypsy moth control (Miller and Lindsay 1993a,b) suggest that gypsy moth infestations are regarded as highly undesirable by the general public, both in terms of aesthetic damage and the potential for adverse effects on human health. Among 629 individuals residing in infested areas, the most frequent reasons for a willingness to pay for control measures against the gypsy moth were aesthetic damage (15%) and

the nuisance factor (13%) (Miller and Lindsay 1993b). Concerns regarding adverse health effects directly related to exposure were expressed by 4% of the responders.

In most instances, gypsy moth defoliation will have little effect on adjacent water bodies (Corbett and Lynch 1987; Grace 1986). During heavy infestations, water quality may be affected by increased runoff and by direct contamination with frass. During active defoliation, fecal streptococci levels in stream water were as high as 25,000/100 mL and fecal coliform densities exceeded 90/100 mL (Corbett 1991). Long term studies of the impact of gypsy moth defoliation on water quality have included studies that show stream water chemical concentrations following defoliation that have included increasing amounts of strong acid anions, base cations and hydrogen ions, as well as decreasing concentrations of acid neutralization capacity (Webb et al. 1995). In addition dissolved nitrogen as nitrate will increase in streams following gypsy moth defoliation (Eshleman et al. 1998).

There are neither studies that directly address the contamination of water with frass nor reports in the literature of adverse effects on human health associated with water contamination from frass. In gypsy moth defoliated forests, however, frass output reached 756 kg (dry weight)/ha in a 1 month period (Grace 1986).

Lyme disease, which is a bacterial infection induced by *Borrelia burgdorferi*, causes serious health effects in humans. In the northeastern and central United States, the primary vector is the black-legged tick, *Ixodes scapularis* (CDC 2004). The tick can infect the white-footed mouse, *Peromyscus leucopus*, and ticks from the white-footed mouse can infect deer or humans (Ostfeld 2002; Ostfeld et al. 1996). As discussed in Section 4.1.2.1, the white-footed mouse eats acorns produced by oak trees. Gypsy moth infestations or outbreaks may result in decreases in acorn production due to damage to oak trees, which, in turn, may cause decreases in the population of white-footed mice due to decreases in food abundance (Elkinton et al. 1996, 2002). There is speculation that this decrease in the population of mice may limit the transmission of Lyme disease to humans due to the adverse effect on the primary vector (Jones et al. 1998; Randolph 1998). Currently, however, there does not appear to be sufficient information to assess the plausibility of this supposition.

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview.

The number of larvae per unit area or tree might be considered the most direct and relevant measure of human exposure because it is contact with the larvae that causes skin irritation, the adverse effect typically associated with the gypsy moth. The available dose response data, however, are based on studies in which exposure is quantified as the number of eggs masses per acre and thus this is the exposure measure that is used in this risk assessment. As long as gypsy moth populations remain sparse, the larvae usually eat only a small proportion of the foliage of even their most favored host species, and contacts with people are rare. In such cases, egg masses generally do not exceed 50 egg masses/acre. During full-scale outbreaks, densities of about 5000 egg masses/acre are common and densities greater than 20,000 egg masses/acre are occasionally recorded.

3.2.2. Exposure Metameter.

Gypsy moth populations can be monitored by estimating the numbers of egg masses (typically expressed as egg masses per acre), the number of larvae (which can be expressed as larvae or larval mass per unit area or larvae per tree), or the number of adults per unit area. For adult moths, population surveys usually involve the use of pheromone traps with or without an insecticide. Surveys of larval populations may involve band trapping, direct examination, or correlations between frass volume and population density. Measurements of larval populations can be highly variable over time and among different species of trees. For example, Naidoo and Lechowicz (2001) conducted larval counts on different species of trees in a deciduous forest in Quebec. In preferred tree species (i.e., red oak), larval populations were as high as 250 larvae per tree. In less preferred tree species, larval populations were much lower, ranging from about 4 larvae per tree (white ash) to 10 larvae per tree (sugar maple). In terms of larval mass, values of 8.4 kg/ha in the month of June and 16 kg/ha in the month of July were measured during severe infestations (Grace 1986).

While the number of larvae per unit area or tree might be considered the most direct and relevant measure of human exposure, the available dose response data (Section 3.3) are based on studies in which exposure is quantified as the number of eggs masses per acre (i.e., Tuthill et al. 1984; O'Dell 1994). As long as gypsy moth populations remain sparse, the larvae usually eat only a small proportion of the foliage of even their most favored host species, and contacts with people are rare. In such cases, egg mass densities generally do not exceed 50 egg masses/acre.

For several years, gypsy moth populations may exist in a density range high enough (between 50 and 500 egg masses/acre) to make the insect a minor nuisance in wooded communities and cause partial defoliation. Once, however, the gypsy moth population increases to a full-scale outbreak, the combination of insect frass and leaf fragments, loss of shade at midsummer, and the large number of larvae may become a major nuisance (Williams 1982). Although the duration of such outbreaks is unpredictable, the principal factors that influence the pest include a variety of pathogens, intraspecific competition for food, and inclement weather (Campbell 1981; Podgwaite

1981; Miller et al. 1989). During full-scale outbreaks, densities of about 5000 egg masses/acre are common and densities greater than 20,000 egg masses/acre are occasionally recorded.

Egg mass densities in infested areas tend to be lower in areas where the human population is dense, compared with less densely populated areas. At the forest periphery, however, egg mass densities can be much higher and seem to be associated with man-made objects (Campbell et al. 1976). Within a relatively limited geographical range, egg mass densities may vary remarkably. For instance, in a heavily infested area with a mean egg mass density of approximately 3800 egg masses/acre, egg mass counts ranged from 0 egg masses/0.1 acre surveyed to 1000 egg masses/0.1 acre surveyed (O'Dell 1994). Similar variability in egg mass density were observed in larger survey areas, as well (Reardon et al. 1993). During a heavy infestation, as many as 50,000 larvae may inhabit a single tree. At such extremely dense concentrations, the generation of frass may be sufficiently intense to be audible, sounding like a light rain (Beaucher and Farnham 1982).

3.2.3. Intensity of Exposures

Given the localized variability in larval populations, quantitative estimates of exposure to larvae cannot be made. Epidemiology studies conducted in gypsy moth infested communities suggest that larval density as a measure of the intensity may not be meaningful. The most important factor in assessing exposure may be the probability of coming into contact with one or more larvae, rather than the number of larvae in a population. In this respect, patterns of human behavior, such as the amount of time spent outdoors and certain kinds of activities likely to result in contact with larvae may be more important than measurements of the local larval population. The likelihood of human exposure to the gypsy moth is likely to increase in proportion to the increases in the larval population in a given area; however, it is not possible to estimate more precise relationships of larval population density to human exposure.

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview

The dose-response assessment for human health effects is based on reports of skin irritation in two populations: one with low exposure (an average of 32 egg masses/acre) and the other with high exposure (an average of 3809 egg masses/acre) (Tuthill et al. 1984). The low-exposure group exhibited no increase in skin irritation and 32 egg masses/acre is taken as a NOAEL for humans and is used as a surrogate RfD for exposure to the gypsy moth in a manner analogous to the use of RfD values for control agents. The high exposure group did evidence a significant increase in skin irritation. Based on the observed dose-response relationship, egg mass densities up to 128 egg masses/acre are not likely to cause a detectable increase in skin irritation or rashes. In addition to these quantitative estimates, the severity of the response is important, particularly in a comparison of effects caused by exposure to the gypsy moth and effects caused by exposure to the agents used to control the gypsy moth. Dermal responses to the gypsy moth are sufficiently severe to have generated numerous case reports. While precise statistics are not available, it does appear that the severity of the skin irritation is sufficient to cause appreciable numbers of affected individuals to seek medical care. While exposure to the gypsy moth is associated with irritation to the eyes and respiratory tract, quantitative dose-response relationships for these endpoints cannot be developed.

3.3.2. Effects on Skin

Of the several available studies that demonstrate skin irritation in humans after exposure to gypsy moth larvae (see Section 3.1.3), the study by Tuthill et al. (1984) is the most appropriate from which to derive a quantitative dose-response assessment. Tuthill et al. (1984) investigated adverse dermal responses in many individuals after exposure to the gypsy moth in areas of high and low infestation. As summarized in Table 3-1, the most relevant data are taken from two groups, one that consisted of 557 individuals in an area of low infestation (Medway, Massachusetts) and one that consisted of 508 individuals in an area of high infestation (Lunenberg, Massachusetts). Although the survey was conducted in the summer of 1982 over two time periods, prior to and after larval emergence, the exposure estimates are based on egg mass counts taken in the fall of 1981. In the Tuthill et al. (1984) publication, the egg mass counts are given only as ranges: 10 to 10,000 egg masses/acre in the area of high infestation and 0 to 70 egg masses /acre in the area of low infestation.

One of the coauthors of the Tuthill et al. (1984) study surveyed egg masses in the two communities (O'Dell 1994). In the high exposure community, surveys were conducted on 27 0.1-acre plots throughout the infested area between October 5 and 6, 1981. In the high exposure areas (Lunenberg), the average egg mass density was 3809 egg masses/acre. In the low exposure community (Medway), 20 sites were surveyed. The arithmetic average number of egg masses/acre was 32, but the egg masses were unevenly dispersed. No egg masses were found at 15 of the 20 sites, and egg mass counts at the other 5 sites were 2, 2, 3, 7, and 50. These egg mass counts were made in the fall, before the outbreak of rashes in the following summer. The use of these egg mass densities as a surrogate for estimating exposure to larvae is based on the assumption that there is a positive correlation between the number of viable larvae in the summer

and the number of egg masses in the preceding fall. Occasionally, below normal midwinter temperatures have resulted in high mortality among overwintering eggs (Bess 1961). Usually, however, fall egg mass counts are closely related to subsequent larval density, particularly among early instars. For the dose response assessment, the average egg mass counts are used for each site—i.e., 32 egg masses/acre for Medway and 3809 egg masses/acre for Lunenberg.

In the Tuthill et al. (1984) study, response data for both the low and high exposure areas are presented as the number of individuals with and without signs of dermal irritation. This type of data is typically termed quantal or discrete and can be used to assess the statistical significance of differences between two groups using the Fisher Exact Test (Uitenbroek 1997). Typically, the Fisher Exact Test is used to determine if there are significant differences between a control group and an exposed group. The Fisher Exact Test yields a *p*-value, the probability that the observed difference occurred by random chance. If the *p*-value is very low, the differences are considered statistically significant. Typically, a *p*-value of 0.05 is used as the maximum value for asserting that the differences are significant. If the *p*-value is greater than 0.05, the differences are not regarded as statistically significant.

The Tuthill et al. (1984) study does not include an actual control group—i.e., a population in an area where no gypsy moth were present; however, for both Lunenberg and Medway, the investigators provide responses before and after larval emergence. Consequently, within each group, the response rate prior to larval emergence can be considered a "control" response and the response rate after emergence can be considered a response associated with exposure to the gypsy moth larvae. Using the Fisher Exact Test, the Medway population demonstrates no statistically significant response after larval emergence. In other words, the p-value is 0.3 for the comparison of response rates before and after exposure and the probability that this difference could be due to random variation is 0.3 or 30%. Thus, the exposure estimate of 32 egg masses/acre may be considered a NOAEL (no-adverse effect level). For the Lunenberg population, however, the post-emergence response rate of 50/508 is significantly higher than the pre-emergence response rate of 7/508 and the p-value is 8×10^{-10}. In other words, the probability that the difference is due to random variation is only 8 in 100 million. Thus, the exposure estimate of 3809 egg masses/acre may be considered a LOAEL (lowest observed adverse effect level).

In addition to the pre- and post-emergence dermatological response rates for all individuals in the two areas, Tuthill et al. (1984) also provide post-emergence data on three different age groups: 0-12 years, 13-59 years, and >59 years. Because no pre-emergence response data are provided by age group, no statistical analysis on "control" vs exposed groups can be conducted. Nonetheless, within the high exposure groups (Lunenberg), an age-response pattern is clearly apparent with younger individuals being much more sensitive than older individuals. As indicated in Table 3-1, these differences are both statistically significant and substantial, spanning a nearly 10 fold difference in sensitivity—i.e., 2.1% in older individuals vs. nearly 20% in individuals in the 0-12 year age range.

Whether or not these different response rates in the different age groups represents a true age-specific difference in sensitivity is unclear. Young children, compared with adults, are likely to spend more time out of doors and may be more likely to come into contact with gypsy moth larvae. As indicated in Table 3-1, there is a clear association between the number of individuals who reported touching larvae and the number of individuals who developed a rash after touching larvae. Thus, it is plausible that the age-specific pattern apparent in the data from Tuthill et al. (1984) could be an artifact of greater contact with gypsy moth larvae by younger individuals.

Because Tuthill et al. (1984) included only two exposure groups and no true control group, a more quantitative dose-response assessment is limited. The U.S. EPA (2001) developed a series of models for estimating benchmark doses. As defined by U.S. EPA (2001), the benchmark dose is the estimate of the lower range of a confidence interval for a dose or exposure associated with a defined response rate. For example, a benchmark dose could be calculated as the 95% lower limit for an exposure associated with a 10% response.

Using the U.S. EPA (2001) benchmark dose software, benchmark doses for both 1% and 10% responses were calculated for all groups combined as well as for each age-group. Because of the limited exposure data, the simple exponential model is used:

$$P = 1 - \exp(\beta * EM)$$

where β is the potency parameter in units of proportion responding per egg mass/acre and EM is the number of egg masses/acre. These analyses are summarized in Table 3-1. For all groups combined, the pre-exposure responses (Table 3-1) were used as zero exposure or control responses. For the age-group specific modeling, no control group was used—i.e., the model has zero degrees of freedom. Thus, the *p*-values shown in Table 3-1 are just an indication of whether or not the potency parameter was significantly different from zero.

The results of the dose response modeling are qualitatively consistent with the use of the simpler Fisher Exact Test. The potency parameter is greatest in the 0- to12- year-old groups. The dose-response relationship for the >59-year-old group is not statistically significant (p=0.15), indicating no substantial response in individuals more than 59 years old in either Lunenberg or Medway. Based on the most sensitive individuals, egg mass densities of 128 egg masses/acre are likely to cause adverse effects in no more than 1%—i.e., a response rate unlikely to be detectable in an epidemiology study. Egg masses of 1336, however, are likely to cause a response rate of at least 10%, which would be detectable in a well-conducted epidemiology study. Again, these results are essentially consistent with the NOAEL and LOAEL values discussed above.

For the current risk assessment, the NOAEL of 32 egg masses/acre is used as a surrogate RfD for exposure to the gypsy moth in a manner analogous to the use of RfD values for control agents. While an uncertainty factor is typically applied to NOAEL values to estimate an RfD, no uncertainty factor is used for this risk assessment. This approach seems reasonable based on the

benchmark dose modeling which indicates that egg mass densities up to 128 egg masses/acre are not likely to cause a detectable increase in skin irritation or rashes.

In addition to these quantitative estimates, the severity of the response is important, particularly in a comparison of effects caused by exposure to the gypsy moth and effects caused by exposure to the agents used to control the gypsy moth. Dermal responses to the gypsy moth are sufficiently severe to have generated numerous case reports as well as a study by NIOSH (see Section 3.1.3). One of the criteria for judging the severity of any response is whether or not an individual will seek medical attention as the result of an exposure to a particular agent. No precise statistics on seeking medical attention after exposure to gypsy moth larvae are available. Tuthill et al. (1984) have noted that: *"Less than 10 per cent of the sufferers sought medical care."* As discussed further in the risk comparison for these agents, a response rate of 10% is substantially greater than rates for any of the agents used to control the gypsy moth, based on comparable data.

3.3.3. Other Effects

As discussed in Section 3.1, exposure to the gypsy moth is associated with irritation to the eyes and respiratory tract as well as generalized psychological distress during severe infestations. No data, however, are available for developing quantitative dose-response relationships for these endpoints.

3.4. RISK CHARACTERIZATION

3.4.1. Overview

In sparse to moderate infestations—i.e., egg mass densities of <500 egg masses/acre—adverse effects involving skin irritation are not likely to be detectable in populations of exposed humans. Nonetheless, some individuals who come into contact with gypsy moth larvae could develop skin irritation. In heavy gypsy moth infestations—i.e., >500 to 5000 egg masses/acre—adverse skin reactions would be expected in substantial numbers and the effects would likely be sufficiently severe to cause some individuals to seek medical attention. In extreme outbreaks—i.e., >5,000 to 20,000 egg masses/acre— the effects will be qualitatively similar to those of severe infestations but could affect up to about one-third of the population. Heavy infestations or extreme outbreaks could cause ocular and respiratory effects in some people but the likelihood of observing these effects cannot be quantified. Similarly, severe infestations are often considered to be a nuisance and cause aesthetic damage to the environment. Both of these factors can lead to stress in some individuals. There is speculation that severe damage to oak forests from gypsy moth infestations might result in a decrease in the prevalence of Lyme disease. This effect of gypsy moth exposure obviously would be viewed as beneficial to human health. Currently, however, there does not appear to be sufficient information to assess the plausibility of this supposition. Young children may be a group at special risk from effects of gypsy moth exposure but it is not clear whether children are more sensitive than adults to the effects of gypsy moth exposure or whether responses in children appear greater because children spend more time outdoors compared with adults.

3.4.2. Effects on Skin

The likelihood of adverse skin reactions in humans after exposure to gypsy moth larvae can be quantified at least in terms of egg mass density (see Section 3.3). Skin irritation also may be considered the most sensitive effect. That is, if exposure levels are less than levels at which a substantial increase in skin irritation is observed, other effects are not likely to be seen.

The risk characterization for the general public is summarized in Table 3-3. The ranges of risk for the general public are based on the ranges of exposure given in column 2 of this table. As in the ecological risk assessment, the stratification of sparse to extreme infestations in terms of eggs masses/acre is somewhat arbitrary but covers a sufficiently broad range to encompass most egg mass densities that are likely to be encountered —i.e., from 50 to 20,000 egg masses/acre. Egg mass densities of 50 egg masses/acre or less are characteristic of mild infestations that occur in the south central region of the United States (Davidson et al. 2001). Egg mass densities of 20,000 egg masses/acre or more are uncommon but can occur in localized areas during gypsy moth outbreaks (Hajek 1997).

Three types of risk characterizations are provided in Table 3-3. The first is based on the NOAEL of 32 egg masses/acre. As discussed in Section 3-3, this value is used as a surrogate RfD for exposure to the gypsy moth. As with all hazard quotients (HQs) based on an RfD, an HQ of less than one indicates that no adverse effects are plausible. The second type of risk characterization is based on a LOAEL of 1336 egg masses/acre. As indicated in Table 3-2, this value is the

estimated benchmark dose associated with a 10% response in the most sensitive subgroup (children < 13 years old). This value is considered a LOAEL rather than a NOAEL because a response rate of 10% would be detected in an epidemiology study and because the value is very close to the observed LOAEL of 3809 egg masses/acre in the study by Tuthill et al. (1984). The interpretation of the hazard quotients based on this LOAEL is different from standard hazard quotients based on an NOAEL or RfD—i.e.,values greater than 1 indicate that adverse effects are likely to be observed in the exposed population.

In addition to the risk characterizations based on the NOAEL/RfD and LOAEL, the last column in Table 3-3 gives the upper range of extra risk associated with each of the exposure categories. These values are derived from the U.S. EPA (2001) benchmark dose software using the one-hit model, as discussed in Section 3.3.

Taken together, all three numerical expressions of risk lead to a consistent qualitative risk characterization. In sparse to moderate infestations—i.e., egg mass densities of <500 egg masses/acre—adverse effects involving skin irritation are not likely to be detectable in populations of exposed humans. Nonetheless, some individuals who come into contact with gypsy moth larvae could develop skin irritation. In heavy gypsy moth infestations, defined in Table 3-3 as ranging from >500 to 5000 egg masses/acre, it is likely that adverse skin reactions will be reported and that the effects will be sufficiently severe to cause some individuals to seek medical attention. In extreme outbreaks—i.e., >5,000 to 20,000 egg masses/acre— the effects will be qualitatively similar to those of severe infestations but could affect up to about one-third of the population.

3.4.3. Other Endpoints

As discussed in the hazard identification (see section 3.2), exposure to gypsy moth larvae is associated with ocular and respiratory effects in humans. In addition, infestations are often considered to be a nuisance and cause aesthetic damage to the environment. Both of these factors can lead to stress in some individuals. Also during severe infestations, water quality may be affected. While all of these concerns may be qualitatively associated with exposure to the gypsy moth and while the severity of these effects are likely to increase with the increasing severity of gypsy moth infestations, no quantitative dose-response assessment can be made (see Section 3.3.3). Accordingly, no quantitative risk characterization can be provided.

As discussed in Section 3.1.5, there is reason to speculate that severe damage to oak forests could result in a decrease in the prevalence of Lyme disease. This effect of gypsy moth exposure obviously would be viewed as beneficial to human health. Currently, however, there does not appear to be sufficient information to assess the plausibility of this supposition.

3.4.4. Sensitive Subgroups

Young children may be a group at special risk from effects of gypsy moth exposure. Although this is suggested in the study by Tuthill et al. (1984) as well as by studies on school children affected by gypsy moth infestations (Aber et al. 1982; Anderson and Furniss 1983), it is not clear

whether the finding indicates that children are inherently more sensitive than adults to the effects of exposure or whether children have a greater incidence of response because they spend more time outdoors than adults and thus have great potential for exposure to gypsy moth larvae.

3.4.5. Connected Actions

There is no evidence to assess the consequences of connected actions involving the various program activities or other common activities associated with the gypsy moth. As discussed in the risk assessment on Gypchek, one of the agents used to control gypsy moths, Gypchek contains gypsy moth parts and may cause irritant effects similar to those caused by the gypsy moth. Consequently, it may be that the effect of simultaneous exposure to gypsy moth larvae and Gypchek would be additive.

3.4.6. Cumulative Effects

Two types of cumulative effects may be considered in assessing the consequences of exposure to the gypsy moth. During an infestation, repeated exposures will occur in the population for the duration over which exposure to the gypsy moth instars occurs. In addition, cumulative effects may be induced from year to year as infestations reoccur. Cumulative effects from exposure to the larvae during a single season are essentially encompassed by the Tuthill et al. (1984) study, the epidemiology study on which the risk assessment is based, because the investigators monitored effects in populations during the period in which early instars were present. The available data do not permit a definitive assessment of the cumulative effects of exposure to the gypsy moth over several seasons. As discussed in the hazard identification (see Section 3.1.2), there is evidence to suggest that an allergic reaction may be one of the mechanisms involved in the dermal effects associated with exposure to the gypsy moth. Thus, it is plausible that some individuals may become sensitized to the gypsy moth after repeated exposures over 1 or more seasons.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview

The clearest primary effect of gypsy moth infestations is on terrestrial plants, primarily trees. Various instars of the gypsy moth larvae will feed on host trees and can cause extensive defoliation which can kill some of the infested trees. On a larger scale, the extensive defoliation and/or death of trees may result in secondary changes to vegetation, which will, in turn, affect other forms of vegetation as well as various animal species (primarily related to changes in habitat). Gypsy moth larvae appear to have definite food preferences; oak, birch, poplar and apple trees seem to be their favorite food sources. While both the European and Asian gypsy moth cause similar types of damage (i.e., defoliation), their feeding preferences are somewhat different with the Asian gypsy moth preferring a wider range of vegetation. Heavy defoliation is much more common among the oaks than among trees that are not particularly favored as food by the gypsy moth. For susceptible oaks, the effects of infestations on tree mortality varies according to the initial condition of the stand and the severity and frequency of defoliations. Generally, gypsy moth infestations result in mortality losses of less than 15% of total basal area. When heavy defoliation is followed by massive overstory mortality, existing shrub and herb cover increase dramatically due to increases in available light, moisture, and nutrients.

The only other group of organisms that are likely to be directly effected by the gypsy moth are some and probably very few other lepidopteran species, including the northern tiger swallowtail butterfly. The mechanisms for direct adverse effects may include bacterial contamination of the leaves by gypsy moth larvae and a decrease in the nutritional value of the leaves damaged by the gypsy moth. Most studies, however, do not indicate substantial direct effects on other insects, including lepidoptera. In some cases, increases may be seen in populations of insect predators of the gypsy moth.

There is no indication in the literature that the gypsy moth will cause direct adverse effects in most groups of animals. Indirect effects, associated with damage to vegetation, may be of substantial consequence to some species, including squirrels, mice, and other mammals that rely on acorns. Although some mammals consume insects, including the gypsy moth, there is no evidence that gypsy moth outbreaks have a substantial impact on insectivorous mammals. Similarly, there is little indication that birds or aquatic species will be adversely affected by the gypsy moth. In some species of birds, gypsy moth infestations and subsequent defoliation may be beneficial, especially for species of birds that favor dead wood as a habitat.

4.1.2. Terrestrial Organisms

4.1.2.1. Mammals – As discussed in Section 3.1 (Human Health Hazard Identification), direct exposure to gypsy moth larvae causes various irritant effects in humans —i.e., skin, eyes, and respiratory tract. In most species of mammalian wildlife, however, fur is likely to reduce the risk of direct contact between the gypsy moth and the skin of the animal, making skin irritation an

unlikely result of exposure. Evidence of irritation to the eyes and or respiratory tract in mammalian wildlife species after direct contact with the gypsy moth is not found in the literature.

Although the hazard identification for the direct effects of gypsy moth exposure in mammalian wildlife is basically negative, indirect effects may be of substantial consequence to some species, as discussed in Section 4.1.2.5. For instance, gypsy moth outbreaks that cause substantial defoliation and mortality in some tree species, particularly oaks, could adversely affect the production of acorns (Gottschalk 1990a; McConnell 1988), which may limit food availability for some forest mammals.

To determine the effects of a gypsy moth outbreak on a population of black bears (*Ursus americanus*), Vaughan and Kasbohm (1993) monitored the behavior of 54 radio-collared black bears in the Shenandoah National Park after a gypsy moth outbreak that caused widespread defoliation, hard mast (acorn) failures, and tree mortality. The outbreak had no apparent effects on cub production or mortality rates of cubs or adults. Although the bears exhibited different habitat preferences at all seasons, they did not avoid defoliated habitat. In the fall, before the gypsy moth infestation, the bears ate mostly acorns. When acorns were no longer available due to defoliation of oak trees by the gypsy moths , the bears switched to eating fruit, which had no apparent impact on the nutritional quality of their diets. Seventy-one percent of bear dens were in tree cavities, primarily in living oaks (mean diameter at breast height = 98 cm). Gypsy moth-induced mortality of den trees was high and by the end of the study, 54% of the living oaks used as dens were dead. While no short-term effects were noted, Vaughan and Kasbohm (1993) speculated that the long-term adverse impact of defoliation on black bears may be a reduction in den sites, with natural replacement possibly requiring 50 years. Conversely, black bears will use as dens the upturned stumps of large dead trees that have been blown over. These would be expected to increase after severe defoliation sufficient to cause tree mortality.

Variations in acorn and other mast production are directly related to variations in populations of squirrels, mice, and other small mammals (Brooks et al. 1998; Gorman and Roth 1989; Nixon et al. 1975). The size of the acorn crop in the fall directly affects the population density of mice living in oak-dominated forests the following spring (McShea and Rappole 1992; McShea and Schwede 1993). By damaging oak tress, gypsy moth infestations can decrease acorn production and a decrease in acorn production secondary to gypsy moth infestations has been shown to decrease the population of white-footed mice, *Peromyscus leucopus* (Elkinton et al. 1996, 2002).

Although some mammals consume insects, including the gypsy moth (see Section 2.4), there is no evidence that gypsy moth outbreaks have a substantial impact on insectivorous mammals. Also, there is no evidence that the effects of gypsy moth outbreaks on other insect populations will directly or indirectly affect mammals that prey on insects. Sample et al. (1996) found no significant effects on the consumption of insects by Virginia big-eared bats in areas of high gypsy moth infestation.

4.1.2.2. Birds – There is little indication that birds will be adversely affected by the gypsy moth. Based on predation by various species of birds on the gypsy moth compared to other hairless lepidoptera, some species of birds appear to avoid the gypsy moth as a prey species (Smith 1985). This suggests, at least indirectly, that the setae or hairs on the gypsy moth larvae may have irritant properties for birds. Direct adverse effects, however, have not been noted in the literature. Reported increases in nesting failures of various species of birds appear to be due to increased predation and/or increased weather stress, both associated with defoliation (Crocoll 1991; Thurber et al. 1994).

In some species of birds, gypsy moth infestations and subsequent defoliation may be beneficial, especially for species of birds that favor dead wood (snags) as a habitat. As a result of defoliation by the gypsy moth, the amount of dead wood increases, particularly in the upper story and the dense vegetation in lower forest strata, providing habitat that is scarce in closed-canopy forests. These secondary effects of gypsy moth outbreaks, which can be considered beneficial to numerous birds, are well documented in the gypsy moth literature (Bell and Whitmore 1997a,b**;** DeGraaf 1987; DeGraaf and Holland 1978; Showalter and Whitmore 2002). Bell and Whitmore (1997) report that available nesting and foraging resources increased for several bird species as result of more snags, windfall, and shrub cover after defoliation, while there was no substantial impact from upper canopy defoliation on birds residing primarily in the forest canopy. Only tree nesting and flycatching guilds appeared to be affected adversely by the moth infestation. Cavity-nesting birds also benefitted indirectly from a gypsy moth outbreak (Showalter and Whitmore 2002). Thurber (1993) noted that bird density increased in plots in which the defoliation was of low to moderate impact. Species richness increased from 19 to 23 species per plot, with declines noted only for tree nesters and flycatchers on high impact plots (Thurber 1993). Increases in low shrub and ground nesters, cavity nesters, low shrub and ground foragers, bark foragers, forest edge species, short-distance migrants, year-round residents, and woodpeckers were widespread, but most pronounced on moderate impact plots. DeGraaf and Holland (1978) reported similar results, finding significantly fewer numbers of only 4 out of 36 bird species examined in heavily defoliated areas. DeGraaf (1987) notes no substantial effects on abundance of various species of birds in defoliated and non-defoliated stands in central Pennsylvania studied over a two year period.

4.1.2.3. Reptiles and Amphibians (Predominantly Terrestrial) – There is very little information regarding the effects of gypsy moth infestations or outbreaks on amphibians or reptiles. In the short-term, gypsy moth defoliation could have a negative impact on some habitats occupied by reptiles and amphibians by increasing solar radiation on dead and down material, litter, and the other materials found above subterranean habitats; however, in the longer term, the defoliation-induced increases in dead and down material will be beneficial to reptiles and amphibians (Schweitzer 1988). Peterson (1990) conducted a field study in south central New York on the effect of gypsy moth infestations on the timber rattlesnake, *Crotalus horridus* and noted that gypsy moth-induced defoliation had an adverse effect on rattlesnakes, primarily through reductions in acorn production and the consequent decrease in the population of small rodents that the snakes eat (see Section 4.1.2.1).

4.1.2.4. Terrestrial Invertebrates – Some lepidopteran species may be adversely affected by gypsy moth outbreaks and at least some of these effects may be direct rather than secondary. Redman and Scriber (2000) examined the adverse effects of the gypsy moth on the northern tiger swallowtail butterfly, *Papilio canadensis*, and demonstrated several different mechanisms associated with the adverse effects. Direct effects included 100% mortality in *Papilio* larvae exposed to leaves painted with gypsy moth body fluids, and 84% mortality in *Papilio* larvae fed leaves from aspen stands infested with gypsy moth larvae. Although the cause of death in the *Papilio* larvae was not clear, the investigators speculate that it was generally due to bacterial contamination of the leaves by gypsy moth larvae, since sterilized leaves did not cause a significant increase in mortality. Moreover, the damage to aspen leaves caused by gypsy moth larvae decreased the nutritional value of the leaves, which led to reduced growth rate and survival of the *Papilio* larvae. In addition, fields studies conducted by Redman and Scriber (2000) demonstrated that proximity to gypsy moths increased the rate of parasitism of the *Papilio* larvae.

The potential adverse effects of gypsy moth outbreaks to lepidoptera was also investigated by Sample et al. (1996) in a study designed to compare lepidopteran populations in 50 acre plots in mixed oak, hickory, and pine forests in West Virginia. Contaminated plots were characterized as stands with average egg mass densities of 235-1156 egg masses/ha (95-468 egg masses/acre), larval abundance of about 68-111 larvae/50 g dry foliage, and defoliation rates up to 88% over a 3-year period. Uncontaminated plots were characterized as stands with average egg mass densities of about 15-180 egg masses/ha (6-72 egg masses/acre), larval abundance of about 4-18 larvae/50 g dry foliage, and no defoliation over a 2-year period with 40% defoliation in the third year. Decreases in abundance and richness of larvae and adults from the family Arctiidae (tiger moths) were apparent in plots infested with gypsy moth larvae, compared with uncontaminated plots. The differences were statistically significant for both abundance (p=0.038) and species richness (p=0.0015). No substantial differences were observed in other lepidoptera or other invertebrate taxa, although a significant increase was noted in braconid wasps. Sample et al. (1996) suggested that the increased abundance of braconids in the plots with gypsy infestation was likely due to increased host (i.e., gypsy moth) availability.

The study by Work and McCullough (2000) demonstrates further that the impact of the gypsy moth is negative to only a small proportion of the lepidopteran community, primarily species that feed on oak and for which the larval development of the affected species and gypsy moth presumably coincide. Although the study does not address the mechanism(s) by which the gypsy moths adversely affect the lepidopteran community, the investigators suggest they might include altered host/plant quality, increases in natural enemies, or microclimate changes. All but the latter mechanism are demonstrated in the study by Redman and Scriber (2000) discussed above. No significant effects were observed on generalist woody plant feeders. Summerville and Crist (2002) criticize the guild classification used by Work and McCullough (2000); however, it is not clear what impact the use of alternate guild classifications would have on the conclusions reached in the study.

Contrary to studies demonstrating the adverse effects of gypsy moth infestations to some macrolepidoptera, anecdotal reports suggest that certain lepidopteran species respond positively to gypsy moth infestations. Schweitzer (1988) claims that 1981 produced the highest number of butterfly species ever for the New Haven, Connecticut area, which for many years stood as the record for eastern North America, despite the record number of acres defoliated by the gypsy moth that same year.

4.1.2.5. Terrestrial Plants (Macrophytes)

4.1.2.5.1. Gross Effects on Trees – The clearest primary effect of gypsy moth infestations is on terrestrial macrophytes, primarily trees. Various instars of the gypsy moth larvae will feed on host trees and can cause extensive defoliation which can kill some of the infested trees. On a larger scale, the extensive defoliation and/or death of trees may result in secondary changes to vegetation, which will, in turn, affect other forms of vegetation with consequences to various animal species (primarily related to changes in habitat).

Trees that are defoliated by even 75% or more are likely to refoliate during the same season. The refoliated leaves are smaller and fewer, and repeated defoliations can cause additional reductions in leaf size (Wargo 1981a). According to Wargo (1981b), trees that refoliate are completely out of phase with the season. Visually, for example, the condition of trees in a mixed composite stand of oaks (red, black, scarlet, and white) in eastern New England showed rapid decline in the year after defoliation and continued to decline slightly during the next 5 years. Following a single heavy defoliation, about 10 years passed before these trees returned to their predefoliation condition (Campbell and Sloan 1977b).

Parker (1981) identifies five key factors that determine the effects of tree defoliation. The factors include, severity (how much foliage is removed); frequency (the number of successive years of defoliation); timing (when in the growing season the tree is defoliated); pathogens (the presence and number of secondary organisms); and health and vigor (the physiological condition of the tree when it is defoliated). Defoliation appears to have a direct impact on root carbohydrates (Kosola et al. 2001, 2002). Most hardwood (or deciduous) trees are able to tolerate at least 2 years of defoliation before root starch content (useable energy) is depleted (Wargo 1981a). Since most coniferous species store carbohydrate resources necessary to refoliate in the leaves, they are usually unable to survive a single, complete defoliation (Johnson and Lyon 1988). Further decline and possible death of previously defoliated eastern hardwood trees are due primarily to secondary organisms like the shoestring fungus, *Armillaria* species, and the twolined chestnut borer, *Agrilus bilineatus* (Wargo 1981b). The defoliator and borer cause adverse effects in the crown of the tree. The borer affects the main stem and the fungus attacks the roots (Wargo 1977, 1981b). Gottschalk (1994) notes that by removing weak, sickly trees from the forest population, fungus (*Armillaria* species) and tree borers (*Agrilus* species) play an important and positive role in forest health.

Previous stand disturbance, which may allow partial colonization of root systems by *Armillaria*, increases rhizomorph abundance (Twery et al. 1990; Wargo 1989). Even in the presence of

abundant rhizomorphs, however, non-defoliated and lightly defoliated trees may remain resilient (Twery et al. 1990). Stressed trees also provide an environment that favors the survival of the twolined chestnut borer (Twery 1991; Wargo 1977), which is attracted to volatile chemicals released by stressed oaks (Dunn et al. 1986a). The trees most susceptible to the pest are those with low stores of starch reserves; however, only the trees with extremely low winter root starch reserves are likely to die (Dunn et al. 1986b, 1987).

Factors that contribute to interspecies differences in response to defoliation include where in the tree reserve energy is stored, the amount of energy required to refoliate, and how much energy is needed to maintain growth during refoliation (Twery 1991). Hemlock, for example, usually will not survive even one complete defoliation (Stephens 1988), whereas some oaks on dry sites may survive repeated defoliations indefinitely (Houston and Valentine 1977; Bess et al. 1947; Twery 1991).

Heavy defoliation is much more common among the oaks than among trees that are not particularly favored as food by the gypsy moth. For susceptible oaks, the effects of infestations on tree mortality varies according to the initial condition of the stand and the number of infestations. Davidson et al. (1999) found that stands with good crown condition, had mortality rates of only 7% and 22% after one and two infestations, respectively; however, in stands with poor crown quality, the corresponding mortality rates were 36% (one infestation) and 55% (two infestations). Heavy defoliation usually increases mortality rates even among trees that are generally not preferred by the gypsy moth as food sources. For instance, a single heavy defoliation in eastern New England resulted in 69% mortality of trees that are eaten but not preferred by the gypsy moth compared with about 37% mortality in oaks, a source of food that is preferred by the gypsy moth (Campbell and Sloan 1977b).

Gypsy moth infestations generally result in mortality losses of less than 15% of total basal area. For example, in an artificially induced gypsy moth outbreak in poplars (*Populus euramericana*) that resulted in nearly complete (70-100%) defoliation of some stands as well as a 25% decrease in stem production, tree mortality ranged from 6 to 10% (Agrawal et al. 2002). Losses of 15-35% are not uncommon, and losses occasionally exceed 50% (Gottschalk et al. 1987). Volume growth is reduced among surviving trees for approximately 3 years after a defoliation episode (Picolo and Terradas 1989; Twery 1991; Muzika and Liebhold 1999). The study by Twery (1987) indicates that, on average, stem volume growth in oaks decreased 20% in any year in which a tree was defoliated, compared with the previous year in which there was no defoliation. This effect is due in part to the reduced leaf area in the recovering trees (Wargo 1981a). In any given stand, heavy defoliation year after year tends to be a rare event. When such an event does occur, however, consequent tree mortality rates may become very high. In the area described by Campbell and Sloan (1977b), for example, only 7% of the mixed oaks rated "good" died following a single heavy defoliation. After two successive heavy defoliations, however, mortality rates in this category increased to 27%.

Between 1911 and 1921, defoliation caused by the gypsy moth was heavy along the eastern seaboard of New England. During this decade the oak component suffered about 60% mortality. About 30% of red maples and 33% of white pines also died (Campbell and Sloan 1977b). During the next decade, both defoliation and the responses to it were significantly less (Baker 1941; Campbell and Sloan 1977b). Tree mortality in response to gypsy moth outbreaks appears to follow a general pattern in which the most severe tree mortality occurs during and after an initial outbreak (Gansner and Herrick 1984; Herrick and Gansner 1986, 1988; Twery and Gottschalk 1989; Twery 1991). Campbell and Sloan (1977b) observed that certain trees within any given species consistently suffered heavier defoliation than others and were more likely to die, suggesting that differential intraspecific mortality could account for the subsequent decrement in stand vulnerability. Similarly, Byington et al. (1994) noted marked difference in tolerance to gypsy moth damage among nine families of red oak.

4.1.2.5.2. Differential Feeding Preferences for Trees – Gypsy moth larvae appear to have definite food preferences; oak, birch, poplar, willow and apple trees seem to be their favorite food sources. In the northeast, preferences vary among species of oak with the greatest preference shown for black and burr oak (Foss and Rieske 2003). Other species, like beech, maple, and white pine are less favored by the gypsy moth, and hemlock and pitch pine seldom serve as food sources. Mortality in white pine, however, can be substantial in stands where pine occurs in the understory. Much less damage occurs in oak/pine stands where pine shares the canopy with oak (Brown et al. 1988). Other species of trees such as black locust and ash are generally not substantially damaged by the gypsy moth (Campbell 1979). The avoidance of green ash (*Fraxinus pennsylvanica*) by gypsy moth larvae seems to be related to the presence of chemicals—not clearly identified—in the leaves of the trees (Markovic et al. 1996). On the other hand, gypsy moth larvae seem able to adapt to even unsuitable hosts such as the alkaloid rich foliage of locust trees (Lazarevic et al. 2002).

In addition to general host preferences, there appear to be regional differentiations among preferred moth host plants. Oak is the preferred species in the east and quaking aspen is the preferred species in the Midwest (Redman and Scriber 2000). Hornbeam is strongly preferred by gypsy moth larvae in Quebec, but in New England it is only an intermediate host, while gray birch and quaking aspen are both preferred by gypsy moth larvae in New England but are classed as only as intermediate hosts in Quebec (Mauffette et al. 1983).

Compared with the European gypsy moth, the Asian gypsy moth feeds more voraciously and grows faster on white oak, larch, and paper birch. In the former Soviet Union, the Asian gypsy moth feeds on more than an estimated 600 tree or plant species. Moreover, the Asian gypsy moth may not only thrive on the same tree species eaten by the European gypsy moth, but may do better on many species that the European gypsy moth does not favor, such as the Douglas-fir (USDA/FS 1992). Waller (1994) reports that the Asian gypsy moth grows better than European gypsy moth on 50 plant species in the United States, with the greatest differences in growth rates associated with coniferous species (Wallner 1994). At least with the Asian gypsy moth, drought may be a predisposing factor to severe damage from infestations (Koltunov and Andreeva 1999).

The Asian strain of gypsy moth is a more serious problem in western forests, compared with the European strain (Montgomery 1993).

4.1.2.5.3. Effects on Stand Structure and Productivity – Subdominant trees (trees growing largely in shaded areas) suffer much higher rates defoliation induced mortality compared with the taller dominants after heavy defoliation (Brown et al 1988; Campbell 1979; McGraw et al. 1990; Quimby 1993). Usually, heavy and repeated defoliation results in a more one-storied stand. Although oak growing-stock volume in trees less than 10 inch diameter at breast height actually decreased between 1965 and 1989, the losses were offset by gains in larger trees (Gansner et al. 1993).

Hix et al. (1991) reported increases in red maple, which corresponded with decreases in oak trees, after defoliation in Appalachian forests. Moreover, total density increased from approximately 42,000 to 62,000 stems per acre, which the investigators attributed to the increase in light, nutrients, and moisture reaching the forest floor after defoliation. Regeneration in the Allegheny Mountain region is dominated by red maple, while red maple and birch dominate the Ridge and Valley regions. Oak reproduction is sparse and seedlings are small, compared with red maple and non-commercial seedlings, in the Allegheny Mountains. According to Allen and Bowersox (1989), only 4-16% of the stems were northern red oak or white oak. In many heavily defoliated stands, oak reproduction, which greatly depends on the survival of acorns and small oak seedlings (0-1 ft tall) is a major concern, especially given the limited information regarding the ability of oak to compete successfully with birch and maple trees (Twery 1991; Galford et al. 1993; Hix et al. 1991).

Moderately heavy defoliation usually accelerates forest succession toward more shade-tolerant (and less defoliation-prone) species (Campbell and Sloan 1977b; Houston 1981b). In contrast to widespread scarcity of oak regeneration in other infested areas, oaks often continue to dominate stands in frequently defoliated areas with excessively drained, sandy soils (e.g., Cape Cod, MA, and the New Jersey coastal plain) or rocky, shallow, ridge top soils (e.g., those common to Medford, MA). Other sources indicate that a small number of oaks in young stands in central New England may become dominant when the stands reach 50 years of age (Oliver 1978; Twery 1991).

Changes in forest composition may account for the frequently-cited reductions in gypsy moth-induced effects in areas such as New England. Gottschalk (1994) reports that moderate to heavy overstory mortality in recent years followed heavy defoliation on about 5-20% of defoliated Appalachian stands. Nevertheless, tree mortality rates in these stands show no indication of downturn after a second wave of equally heavy defoliation.

Even in healthy stands with little defoliation, heavy crops of hard mast (primarily acorns) are only produced about every 3 or more years and during intervening years, mast crops may be poor or nearly absent (USDA/FS 1994). Defoliation can virtually eliminate oak mast production, especially in the short-run and result in several consecutive years of complete mast failure

(Gottschalk 1990b; Liscinsky 1984). During years of moderate and heavy defoliation, short-term and residual adverse effects on mast production can be attributed to three sources: direct consumption of flowers; abortion of immature acorns due to a low carbohydrate supply; and lack of flower bud initiation. Available data suggest that abortion of immature acorns is the most significant of the adverse effects, which can result in up to 5 years of complete acorn failure (Gottschalk 1990a).

As previously noted, trees that do not die during a defoliation episode may take as long as 10 years to recover their full vigor. On the other hand, once trees recover their vigor, significant overstory mortality (>60% of the basal area) in intermediate and suppressed trees (i.e., not heavy mast producers) is required to cause significant reductions in acorn production. Acorn production by surviving trees may even be stimulated by this thinning (Gottschalk, 1990a). In the long term, loss of acorn and other nuts is partially compensated by an increase in the number of flowers (Gottschalk 1990b).

4.1.2.5.4. Effects on Shrubs and Herbs – When heavy defoliation is followed by massive overstory mortality, existing shrub and herb cover increases dramatically due to increases in available light, moisture, and nutrients. Under certain conditions, heavy defoliation and subsequent overstory mortality can result in dominance by shrubbery and herbaceous vegetation for several years. Gansner (1985) describes an understory 10 years following defoliation as being dominated by blueberry, witch-hazel, raspberries, and several species of ferns, along with some tree seedlings that were heavily browsed by deer. Hix et al. (1991) also noted that blueberries and raspberries were often the shrub species that increased the most following defoliation. Among herbaceous plants, Brackley (1985) noted that gypsy moth-induced defoliation appeared to stimulate flowering in the endangered orchid, *Isotria meleoloides*, in New Hampshire.

4.1.2.5.5. Effects on Fire Hazard – Researchers generally agree that heavy defoliation caused by the gypsy moth increases fire danger, although differences in fuels have not been measured and the increased fire hazard has not been calculated (Gottschalk 1990b). Wildfires are more difficult to control in areas of extensive tree mortality (Tigner 1992). Furthermore, the numerous standing dead snags may act as lightning rods and further increase risk of fire starts by lightning. Fire caused by a lightning strike on one or more of these snags could smolder for several days prior to detection (USDA/FS 1994). On the other hand, fires are infrequent during the growing season in eastern hardwood forests. Consequently, significant increases in fire hazard would occur in hardwood forests during the growing season only as the result of long-term increases in woody fuels due to tree mortality (Gottschalk 1990b).

4.1.2.6. Soil and Terrestrial Microorganisms – There is little information from which to directly assess the effect of gypsy moth infestations on terrestrial microorganisms. Indirect evidence suggests that adverse effects are unlikely. Soil microbial activity is largely influenced by moisture and temperature. Vaughan and Kasbohm (1993) report that defoliation increases maximum daily temperatures. Since microbial activity increases with temperature, defoliation is

likely to increase microbial activity, thereby accelerating the process of decomposition. Decomposing bacteria and fungi have high nutrient requirements. Increased nutrient content in litter fall might enhance decomposition, which might be the case during gypsy moth defoliation in the spring when leaf matter is consumed before nutrient reabsorption takes place (Grace 1986). The effects of these increased nutrient levels and mineralization might be to enhance forest regeneration.

4.1.3. Aquatic Organisms.

4.1.3.1. Fish – Little information is available regarding the effects of gypsy moth infestations on fish populations. Defoliation from the gypsy moth can result in an increase in the pH and temperature of ambient water (Downey et al. 1994; Webb et al. 1995). Downey et al. (1994) have suggested that trout, which as are very sensitive to changes in pH and temperature, could be adversely affected by such changes. As discussed by Webb et al. (1995), however, no direct data are available on the biological effects of such changes due to defoliation by the gypsy moth and it is unclear if significant biological effects on fish are likely.

4.1.3.2. Aquatic Invertebrates – Hutchens and Benfield (2000) detected an apparent increase in the rate of leaf breakdown in streams due to gypsy moth defoliation, which might result in food deficits during spring for shredders—i.e. caddisflies, stoneflies, and some dipterans. The number of shredders collected by Hutchens and Benfield (2000), however, was greater in disturbed streams (i.e., streams in areas of gypsy moth defoliation) than in control streams.

4.1.3.3. Aquatic Plants – Information directly related to the effects of gypsy moth infestations on algae in streams is available in the study by Sheath et al. (1986), which was conducted in a spring-fed, headwater stream in central Rhode Island from 1979 to 1982. In the first two summers, a dense riparian canopy reduced surface light penetration to a range of 5-18% of incident radiation, and the stream macroalgal community consisted of only one to four species covering from less than 1 to 35% of the stream bottom. In 1981, the surrounding leaf canopy was removed by a massive gypsy moth larval outbreak, which increased light penetration at the stream surface to 73% and increased the water temperature by 3.7°C. By early August, macroalgal cover increased to a peak of 80% of the stream bottom. A less severe gypsy moth defoliation in 1982 that did not have a significant impact on light, temperature or macroalgal cover from 1979 and 1980. In contrast, investigators on a stream in Shenandoah National Park observed no significant changes in periphyton abundance due to defoliation. These investigators speculated that many southern Appalachian streams are so low in nutrients that increased sunlight penetration alone is not enough to increase algal growth (USDA/FS 1994).

4.1.3.4. Aquatic Microorganisms – Particularly in small, first-order streams, defoliation by the gypsy moth provides increased sunlight at the water surface that may enhance microbial activity secondary to an increase in temperature (Sheath et al. 1986). As discussed in Section 4.1.3.2, defoliation by the gypsy moth appears to increase the rate of leaf breakdown in streams, which is due, in part, to greater microbial conditioning in leaf packs (Hutchens and Benfield 2000). Other major increases occur in fecal coliform and fecal streptococci densities in streams during periods

of maximum defoliation (Corbett and Lynch 1987). These increases might be associated with increases in nutrients from water contamination by frass and leaf fragments.

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview

As in the human health risk assessment, the exposure metameter is dictated by the data used to formulate the dose-response assessment. Also as in the human health risk assessment, egg mass density is the exposure metameter for terrestrial invertebrates and plants because it is the measure on which the dose-response assessment is based. Egg mass densities spanning a range from 5 egg masses/acre to 5,000 egg masses per acre are used to estimate responses in terrestrial plants and invertebrates.

Most wildlife species are not affected directly by exposure to the gypsy moth but are more likely to experience indirect effects like changes in habitat or other environmental conditions secondary to defoliation. Consequently, the exposure assessment for most wildlife species is almost identical to the dose-response assessment for terrestrial plants which is expressed as defoliation caused by gypsy moth larvae. For this exposure assessment, categories of defoliation are defined normal background defoliation (<30% defoliation), moderate defoliation (30-60% defoliation), and high or severe defoliation (>60% defoliation).

4.2.2. Direct Exposure

As discussed in Section 4.1, the gypsy moth has a direct impact on terrestrial vegetation and certain terrestrial invertebrates, and the direct effects, especially the effects on vegetation, are likely to cause indirect effects in other organisms (Section 4.2.3). Like the exposure assessment for human health (Section 3.2), the exposure assessment for terrestrial vegetation and terrestrial invertebrates can be based on any of several exposure measures, including numbers of egg masses per acre, numbers of larvae per acre or tree, or larval mass per acre or tree. The various exposure measures are not necessarily correlated and may relate to more than vegetation damage.

Sample et al. (1996) assayed egg mass density, number of larvae per 50 g dry weight of vegetation, and defoliation in sets of stands: control stands with no significant level of gypsy moth infestation and no pesticide, stands with significant levels of gypsy moth infestation and no pesticide, stands with both gypsy moth infestation and treatment with *B.t.k.*, and stands treated with *B.t.k.* in the absence of significant gypsy moth infestation. Each set of stands consisted of six replicates of 50-acre plots in which measurements were made over a 3-year period. As illustrated in Figure 4-1, the relationship between egg mass density and the number of larvae per unit vegetation is extremely weak and scattered. More recently, Naidoo and Lechowicz (2001) published the results of a 13-year study in which they assayed the number of gypsy moth larvae per tree in different tree species in a forest in Quebec. Figure 2 in the study shows substantial variation in the numbers of larvae in different tree species in the forest. Red oak trees were the most heavily infested (up to 250 larvae per tree), and white ash trees were the least infested (maximum of four larvae per tree). This study clearly illustrates that gypsy moth larvae feed preferentially on different types of vegetation, resulting in substantial variation in infestation among tree species. Although larval density may be the most intuitively reasonable measure of exposure (i.e., to the gypsy moth larvae), the poor correlation of egg mass density to larval density noted in the study by Sample et al. (1996) may be due partly to larval feeding preferences

or dispersal. A complicating factor, discussed further in Section 4.3, is that larval counts themselves do not necessarily predict defoliation uniformly across different species of trees.

As in the human health risk assessment, the exposure metameter is dictated to some extent by the data used to formulate the dose-response assessment. In the human health risk assessment, the dose-response assessment is based on egg mass density (see Section 3.3); therefore, egg mass density is, by definition, the exposure metameter. As discussed in Section 4.3 and summarized in Table 4-1, egg mass densities spanning a range from 5 egg masses/acre to 5,000 egg masses per acre are used to estimate responses in terrestrial plants and invertebrates.

4.2.3. Indirect Exposure

As summarized in Section 4.1, most wildlife species are not affected directly by exposure to the gypsy and are more likely to experience the effects of indirect exposure like changes in habitat or other environmental conditions secondary to defoliation. Consequently, the exposure assessment for most wildlife species is almost identical to the dose-response assessment for terrestrial plants (Section 4.3) – i.e., the assessment is expressed as defoliation caused by gypsy moth larvae. Defoliation can be categorized various ways, all of which are largely judgmental. For example, Agrawal et al. (2000) define light defoliation as 20-40%, severe defoliation as 40-90%, and nearly complete defoliation as 75-100%. The categories used in the previous EIS as well as in the study by Gottschalk et al. (1998) are used in the current risk assessment: normal background defoliation (<30% defoliation), moderate defoliation (30-60% defoliation), and high or severe defoliation (>60% defoliation).

The use of these somewhat arbitrary categories has only a minimal impact on the current risk assessment, which is largely qualitative since the available data do not permit quantitative measures of response as a function of defoliation for most wildlife species. This issue is discussed further in the risk characterization (Section 4.4).

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Overview

The quantitative dose-response assessment for the gypsy moth is illustrated in Figure 4-2. As in the human health risk assessment, the dose metameter is egg masses/acre. Quantitative dose-response assessments can be made for both terrestrial plants and sensitive species of lepidoptera. The dose-response assessments for terrestrial plants are based on a relatively simple quantitative model for the relationship of egg mass density to defoliation. Three broad categories (sensitive, intermediate, and tolerant) are used to characterize the susceptibility of forest stands to gypsy moth induced defoliation. Estimated LOAEL values based on 30% defoliation, which is considered the lower range of moderate defoliation, are approximately 125, 1000, and 7000 egg masses/acre for sensitive, intermediate, and tolerant forest stands, respectively. The corresponding NOAEL values, defined as 10% defoliation, are estimated as 12, 20, and 125 egg masses/acre for sensitive, intermediate, and tolerant forest stands.

The gypsy moth may affect some sensitive terrestrial invertebrates, including some species of lepidoptera. These effects, however, are less well documented and less well characterized compared with the effects on terrestrial plants. Nonetheless, available studies indicate that the NOAEL for adverse effects in certain other species of lepidoptera are lower than the NOAEL for sensitive forest stands—— i.e., about 6-72 egg masses/acre for some lepidoptera.

No quantitative dose-response assessment is presented for other groups of organisms—e.g., mammals, birds, and soil or aquatic organisms. The impact of gypsy moth exposure on these species is most likely to result in indirect effects (i.e., secondary to defoliation). Qualitative expressions of risk for these species are presented in the Risk Characterization (Section 4.4.4).

4.3.2. Terrestrial Plants

In terms of assessing direct effects of the gypsy moth on terrestrial vegetation, the most common endpoint used to express damage is defoliation. As discussed in Section 4.1.2.5, numerous factors, many of which are interrelated, influence the level of damage that gypsy moth larvae may cause to a forest stand or region. Several models are useful for quantifying the likely levels of defoliation (e.g., Davidson et al. 2001; Gansner et al. 1985; Gottschalk et al. 1998; Gribko et al. 1995; Liebhold et al. 1993; Montgomery 1990;Weseloh 1996a; Williams et al. 1991). Furthermore, the USDA Forest Service developed an expert system, GypsES, to facilitate the use of modeling in the management of gypsy moth programs (Gottschalk et al. 1996; Williams et al. 1997; http://www.fs.fed.us/na/morgantown/fhp/gypses /gypmain.htm).

The common exposure factor for all of these models is egg mass density. As discussed in Section 3.1, egg mass density is usually measured during the fall to predict damage in the following season. This indicator allows individuals involved in gypsy moth control programs enough time to plan an intervention strategy before damage occurs. The models, some of which are extremely complex, incorporate several factors, in addition to egg mass density, that affect defoliation—e.g., types of vegetation, terrain characteristics, and various geographical characteristics. For example, Weseloh (1996a) developed a 23-parameter model that

incorporates egg mass density, latitude, longitude, elevation, drainage, factors for the history of defoliation over a previous 4-year period, and 12 parameters for interaction terms. The model was developed using data from defoliation patterns in Connecticut from 1987 to 1994. When used retrospectively on defoliation rates from 1974 to 1986, the model was reasonably accurate in predicting years of heavy defoliation (correlations of up to 0.8) but less accurate in predicting years with relatively little defoliation (correlations on the order of 0.2).

For the current risk assessment, the relatively simple four-parameter model developed by Davidson et al. (2001) seems most useful for describing key factors that will have an impact on defoliation:

$$DEF_S = (BA_S/BA_T)^{b1} \times BA_p^{b2} \times \ln(EM)^{b3} \times Y \times e^{\gamma Y}$$

where:

Y	=	number of years since start of outbreak
DEF_S	=	percent stand defoliation at time *Y* of outbreak
BA_S	=	basal area of sensitive species in stand (m^2/ha)
BA_P	=	basal area of pine species in stand (m^2/ha)
BA_T	=	total basal area in stand (m^2/ha)
EM	=	egg masses per hectare

and γ, b1, b2, and b3 are model parameters. Based on defoliation and egg count data collected over 4 years (1992 through 1995) from seven pine-oak stands and nine pine-sweetgum stands in Maryland, Davidson et al. (2001) estimated the following values for the model parameters: 0.2226 for b1, -0.2684 for b2, 2.0792 for b3, and -0.5781 for γ. Notably, the parameters associated with the ratio of sensitive tree species (BA_S/BA_T) and egg mass density (EM) are positive indicating that damage increases as these model components increase. The parameters associated with the basal area of pine species (BA_p) as well as the exponential function for years since the start of the outbreak ($e^{\gamma Y}$) are negative. In other words, an increase in the density of pine species (trees generally not favored by the gypsy moth) will tend to reduce defoliation and the outbreak will subside over time. All of these factors in the model are qualitatively consistent with the behavior of gypsy moth infestations (see Section 4.1.2.5).

The model developed by Davidson et al. (2001), though relatively simple, is still multidimensional, which means the estimates of defoliation depend highly on site specific factors. Any number of defoliation estimates could be made based on varying any of the input variables in the model by Davidson et al. (2001). For this risk assessment, three general types of forest stands are considered: sensitive, intermediate, and tolerant. Each stand is assumed to have a total basal area of 15 m^2/ha. This is somewhat arbitrary but since the total basal area is only used as a normalizing factor on the basal area of sensitive species, this assumption has no impact on the model. The basal surface area for sensitive species is taken as 13 m^2/ha for sensitive stands, 6 m^2/ha for intermediate stands, and 2 m^2/ha for tolerant stands. The basal area for pine is taken as 0.25 m^2/ha for sensitive stands, 1.5 m^2/ha for intermediate stands, and 3 m^2/ha for tolerant stands. The percent defoliation in all stands is calculated for 1 year from the initial time

of the outbreak. Egg mass density is modeled over a range from 1 egg mass/ha (approximately 0.4 egg masses per acre) to 25,000 egg masses/ha (approximately 10,000 egg masses per acre). Again, despite the arbitrary nature of these ranges and assumptions, they reflect the variability of and responses among the stands considered in the Davidson et al. (2001) publication.

The variability of responses in the three different stand types is illustrated in Figure 4-2. The curved lines indicate the percent defoliation expected in sensitive, intermediate, and tolerant stands over the range of egg mass densities considered. The two thick horizontal lines represent breakpoints for the classifications of defoliation discussed in Section 4.2.3—i.e., normal background defoliation (<30% defoliation), moderate defoliation (30-60% defoliation), and high or severe defoliation (>60% defoliation). Sensitive stands are likely to show evidence of low level intermediate defoliation—i.e., 30% defoliation—at an egg mass density of about 125 egg masses/acre. Comparable values for intermediate and tolerant stands are about 1000 egg masses/acre and 7000 egg masses/acre, respectively. For sensitive stands, the egg mass density associated with 50% defoliation is about 600 egg masses/acre and this estimate is consistent with field observations for sensitive stands (Montgomery 1990).

Risks to wildlife species from most agents used to control the gypsy moth are based on NOAEL values (no observed adverse effect levels) and LOAEL values (lowest observed adverse effect levels). As discussed in Section 4.2.3, 30% defoliation is used in this risk assessment as a border value between background and moderate defoliation. Thus, the egg mass densities of 125, 1000, and 7000 egg masses/acre for sensitive, intermediate, and tolerant stands, respectively, are essentially LOAEL values—the lowest egg mass density that might be associated with a level of defoliation classified as moderate or a minimal adverse effect.

The model by Davidson et al. (2001) as well as other models for gypsy moth defoliation are non-threshold. In other words, any level of exposure is assumed to carry some risk. Thus, the selection of a functional NOAEL is somewhat arbitrary. Following the general approach used to estimate a benchmark dose (U.S. EPA 2001), the defoliation rate of 10% is used as a functional NOAEL. Based on the dose-response curves illustrated in Figure 4-2, these NOAEL values are egg mass densities of about 12 egg masses/acre for sensitive stands, 20 egg masses/acre for intermediate stands, and approximately 125 egg masses/acre for tolerant stands.

These NOAEL and LOAEL estimates are at best crude approximations of egg mass densities associated with levels of defoliation that might be considered essentially benign (NOAELs) or capable of causing detectable damage (LOAELs) in various forest stands. The primary use of these values is to provide a basis for comparing risks associated with exposure to the gypsy moth to risks associated with exposure to agents used to control the gypsy moth.

4.3.3. Terrestrial Invertebrates

The impact of gypsy moth exposure on terrestrial invertebrates cannot be quantified. As discussed in Section 4.1.2.4, a few relatively recent studies demonstrate that exposure to gypsy moth larvae during an outbreak may adversely affect some other lepidopterans (Sample et al.

1996; Work and McCullough 2000). Furthermore, the study by Redman and Scriber (2000) suggests that at least some of these effects could be related directly to gypsy moth exposure rather than to effects secondary to gypsy moth-induced defoliation.

Although the data on invertebrates are limited in terms of defining a quantitative exposure-response relationship, the study by Sample et al. (1996) defines an apparent NOAEL of 6-72 egg masses/acre for effects on tiger moths. The corresponding LOAEL is 95-468 egg masses/acre and is associated with decreases in the abundance of tiger moth larvae and adults. These values are illustrated in Figure 4-2 as the mean of each range rounded to one significant place—i.e, a value of 40 egg masses/acre for the NOAEL and 300 egg masses/acre for the LOAEL.

4.3.4. Other Species

As discussed in Section 4.2.3, other species may be affected by gypsy moth infestations secondary to defoliation. These observations, as summarized in Section 4.1 (Hazard Identification), are essentially qualitative—i.e., the effects were observed in the field or are based on plausible assumptions in cases of severe gypsy moth outbreaks and extensive defoliation. Thus, no quantitative dose-response assessment is proposed for species that are indirectly affected, and the plausible responses are discussed qualitatively in the risk characterization (Section 4.4.4).

4.4. RISK CHARACTERIZATION

4.4.1. Overview

The best documented and most obvious effect of the gypsy moth will be defoliation of terrestrial vegetation, particularly in forest stands in which sensitive species of trees predominate. In some respects, the risk characterization for terrestrial vegetation is essentially a restatement of the hazard identification. In other words, the effects of gypsy moth larvae on forests is extremely well documented and relatively well understood. In sensitive forest stands—i.e., stands in which oak, birch, and other favored species predominate —gypsy moth larvae can cause substantial defoliation. In forest stands in which tree species that are not favored by gypsy moth larvae predominate —e.g., hemlock, various types of pine, black locust and white ash—even relatively high exposures, as measured by egg mass density, may not result in substantial defoliation. The risk assessment for direct effects on forests should be at least qualitatively influenced by the current range of the gypsy moth, which has not yet extended to some forests in the southeast that may be among the most sensitive to the gypsy moth. Thus, unless measures to contain the gypsy moth are successful, these southeastern forests may suffer serious damage in future infestations.

Some other lepidopteran species also may be directly affected by exposure to the gypsy moth. Most studies, however, suggest that substantial adverse effects in terrestrial insects are unlikely and effects in some insect species, including some other lepidoptera, may be beneficial.

Because the gypsy moth may substantially damage some forests in severe infestations or outbreaks, secondary effects in other species of wildlife are plausible. Reductions in populations of squirrels, mice, and other mammals which may be sensitive to changes in the availability of acorns are likely. Substantial adverse effects on other groups of animals – i.e., birds, reptiles, and aquatic species – cannot be ruled out but have not been convincingly demonstrated.

4.4.2. Direct Effects

4.4.2.1. Terrestrial Plants – A quantitative summary of the risk characterization for forest stands is presented in Table 4-1. Risks are expressed as hazard quotients and estimates of percent defoliation for three classes of forest stands: sensitive, intermediate, and tolerant. As discussed in Section 4.3.2, these classifications are intended to reflect, albeit crudely, differences in susceptibility of various forest stands to the effects of gypsy moth exposure, which is predicated on the feeding preferences of gypsy moth larvae. The specific types of trees favored and not favored by the gypsy moth are discussed in Section 4.1.2.5.2. The NOAEL values and quantitative estimates of defoliation are based on the dose-response model proposed by Davidson et al. (2001). Although the dose-response model is relatively simple, it does appear to reflect the variables that have the most significant impact on the sensitivity of various forest stands to defoliation by gypsy moth larvae. The four categories of infestations used in the dose-response assessment are based on egg mass densities of 5, 50, 500, and 5000 egg masses/acre. These categories generally encompass the range of egg mass densities reported in the literature for infestations ranging in degree from negligible to severe and are similar to the categories used in the human health risk assessment (Table 3-3). The hazard quotients presented in Table 4-1 will be used primarily in the comparative risk assessment, which is a separate document that provides

a quantitative comparison of the risks for each of the various agents used to control the gypsy moth as well as the risks posed by the gypsy moth itself.

In some respects, the risk characterization for terrestrial vegetation is essentially a restatement of the hazard identification. In other words, as discussed in Section 4.1.2.5, the effects of gypsy moth larvae on forests is extremely well documented and relatively well understood. In sensitive forest stands—i.e., stands in which oak, birch, and other favored species predominate —gypsy moth larvae can cause substantial defoliation. In forest stands in which tree species that are not favored by gypsy moth larvae predominate —e.g., hemlock, various types of pine, black locust and white ash—even relatively high exposures, as measured by egg mass density, may not result in substantial defoliation.

The quantitative risk characterization focuses on defoliation; however there are likely to be other effects of gypsy moth exposure. In some cases, extensive defoliation will result in tree mortality. In general, however, tree mortality is likely to be relatively low (on the order of 10-20%), although higher rates of mortality (up to about 50%) are possible when sensitive species are subject to multiple years of defoliation (see Section 4.1.2.5.1). Defoliation and tree mortality can lead to secondary effects of exposure on animals (see Section 4.4.3). Likewise, non-target vegetation may be subject to secondary effects of exposure, such as increases in understory growth (see Section 4.1.2.5.4). The extent to which the secondary effects are considered beneficial or detrimental depends to some extent on forest management objectives which are beyond the scope of this risk assessment.

The risk assessment for direct effects on forests should be at least qualitatively influenced by the range of the gypsy moth. That range has not yet extended to some forests that may be among the most sensitive to gypsy moth exposure. Many forests in the southeast and midwest are populated with a high proportion of tree species that are favored by the gypsy moth. Unless measures to contain the gypsy moth are successful, these regions may suffer serious damage from future infestations by the gypsy moth (Liebhold and McManus 1999).

4.4.2.2. Terrestrial Invertebrates – There is plausible concern regarding direct effects of the gypsy moth on some lepidopteran species. Nonetheless, few studies support this concern relative to the large number of studies regarding effects on terrestrial plants. As summarized in 4.3.3, the study by Sample et al. (1996) suggests a NOAEL of about 40 egg masses/acre for Arctiidae larvae and adults in terms of abundance and species richness. The direct effect of the gypsy moth, however, involved only lepidoptera from a single family, Arctiidae, which includes the tiger moths. No effects were seen in other lepidopteran or non-lepidopteran species; nonetheless, the effects observed on Arctiidae adults and larvae were statistically significant. Based on the approximate NOAEL of 40 egg masses/acre, this family of lepidoptera would still be less sensitive to gypsy moth larvae than most forest stands.

It is difficult to assess the extent to which other lepidopteran or non-lepidopteran groups might be affected by exposure to gypsy moth larvae. Redman and Scriber (2000) report several

mechanisms associated with the adverse effect of gypsy moth larvae on the northern tiger swallowtail butterfly, *Papilio canadensis*. There are, however, no other field studies that suggest the plausibility of substantial adverse effects in most insect species during gypsy moth infestations. In addition, gypsy moth induced defoliation may be beneficial to some butterfly species (Schweitzer 1988) or will have no effect on most other insect species (Sample et al. 1996).

4.4.3. Indirect Effects

4.4.3.1. Terrestrial Mammals – There is only limited information regarding the potential effects of gypsy moth infestations on mammalian wildlife. Adverse effects on reproduction or nutritional status were not observed in black bears after exposure to the gypsy moth during an outbreak that caused substantial mortality in oak trees and decreased acorn production (Vaughan and Kasbohm 1993). As noted in Section 4.1.2.1, bears adjusted for the decrease in acorn production by switching to alternate foods. It is not clear, however, that all mammals would adapt to a severe decrease in hard mast production. Consequently, there is plausible concern about the potential for adverse effects (reductions in populations) in squirrels, mice, and other mammals which may be sensitive to changes in the availability of acorns.

4.4.3.2. Birds – The effects of gypsy moth exposure on birds appear to be highly variable, with reports of both detrimental effects as well as beneficial effects (see Section 4.1.2.2). While the available data are not amenable to quantitative analyses, it would appear that these mixed effects are likely to be most pronounced during severe infestations.

4.4.3.3. Amphibians and Reptiles – There are no studies that clearly indicate adverse effects in either amphibians or reptiles after exposure to the gypsy moth. As discussed in Section 4.1.2.3, substantial defoliation of mast producing trees might adversely affect snakes that consume small mammals (e.g., squirrels and mice), the populations of which might decrease due to mast failure (see Section 4.4.3.1).

4.4.3.4. Terrestrial Microorganisms – There is no information regarding the effects of the gypsy moth or gypsy moth defoliation on terrestrial microorganisms. Intuitively, it seems reasonable that soil microbial activity would increase in response to defoliation as a result of the subsequent increase in ground temperature and nutrient load that would result from increases in litter and frass production (see Section 4.1.2.6). These effects, although not reported in the literature for soil microbial activity, are reported for aquatic microorganisms (see Section 4.1.3.4).

4.4.3.5. Aquatic Organisms – There is very little information to indicate that gypsy moth infestations cause substantial adverse effects on aquatic organisms (see Section 4.1.3). Stream microorganisms are likely to be affected directly by gypsy moth infestations due to the potential increase of microbial activity in forest streams. The increased activity is mostly like due to the increased nutrient loading of streams which results from defoliation and larval frass. Although Hutchens and Benfield (2000) suggest that the increase in activity might cause a food deficit for aquatic insects that shred decomposing leaves, the investigators found that the population of such

organisms (i.e., caddisflies, stoneflies, and some dipterans) were higher in streams in areas of gypsy moth defoliation compared with streams in the control areas. Because of increased light and water temperature secondary to defoliation, algal and aquatic macrophyte growth is likely to be increased (see Section 4.1.3.3), which might increase productivity in some streams but adversely affect water quality and habitat characteristics in other streams.

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Table 3-1. Individuals with skin responses to the gypsy moth in two communities (data from Tuthill et al. 1984 except as noted)

Factor	Medway	Lunenburg
Average egg masses/acre	32 [b]	3809 [b]
All groups combined	#Responding/#Exposed	
Total with rash during week before infestation	6/557	7/508
Total with rash during first 7 days after larvae emerge	9/557	50/508
P-value for pre- vs post-emergence difference [b]	0.30	8×10^{-10}
Subgroups		
Age 0-12 years	2/84 [c] (2.3%)	13/69 [d] (19%)
Age 13-59 years	7/407 [c] (1.7%)	35/342 [d] (10%)
Age > 59 years	0/66 [c] (0%)	2/97 [d] (2.1%)
Larval contact		
Touched larvae	8.3%	31.4%
Rash where individuals were touched or crawled on by larvae	29.0%	82.0%

[a] O'Dell 1994
[b] Based on Fisher Exact Test. See text for discussion.
[c] No statistically significant difference among age groups.
[d] Response in 0-12 years significantly greater than 13-59 year group (p=0.039) and >59 year group (p=0.000245). Response in 13-59 year group significantly greater than >59 year group (p=0.0048). All comparisons based on Fisher Exact Test. See text for discussion.

Table 3-2: Statistical Analyses of Epidemiology Data from Table 3-1.

Age Group	Back-ground	Potency (proportion responding per egg mass/acre)	*p*-value	Lower 95% Confidence Interval on Egg masses/acre	
				1%	10%
0-12 years	0.022	4.89e-005	0.00041	128	1336
13-59 years	0.016	2.40e-005	<0.0001	304	3185
>59 years	0.00	5.52e-006	0.15	697	>11,427
All Groups Combined	0.013	2.37e-005	<0.0001	327	3432

[a] Dose associated with a given extra risk – i.e., 1% or 10%.

Table 3-3. Adverse human health effects for members of the general public associated with exposure to the gypsy moth

NOAEL:	**32**	**egg masses/acre**	
Level of Infestation	Exposure (egg masses/acre)	Hazard Quotient [a]	Upper Limit on Extra Risk [b]
Sparse	50	1.6	1.4%
Moderate	>50-500	>1.6 - 16	>1.4% - 2.5%
Heavy	>500-5000	>16 - 156	>2.5% - 12%
Extreme	>5,000 - 20,000	>156 - 625	>12% - 38%
LOAEL:	**1336**	**egg masses/acre**	
Level of Infestation	Exposure (egg masses/acre)	Hazard Quotient [a]	Upper Limit on Extra Risk [b]
Sparse	50	0.04	Same as above
Moderate	>50-500	>0.04 - 0.4	
Heavy	>500-5000	>0.4 - 4	
Extreme	>5,000 - 20,000	>4 - 15	

[a] Calculated as the exposure in egg masses/acre divided by the NOAEL or LOAEL.
[b] Based on the dose-response model summarized in Table 3-2 using data on all groups combined.

Table 4-1: Summary of quantitative risk characterization for forest stands.

	Forest Stands		
	Sensitive Stands	Intermediate Stands	Tolerant Stands
NOAEL	12	20	125
Egg masses/acre		Hazard Quotients [a]	
5	0.4	0.25	0.04
50	4	2.5	0.4
500	40	25	4
5,000	400	250	40
		Percent Defoliation [b]	
5	5.3%	2.8%	1.8%
50	21.0%	11.0%	6.9%
500	46.0%	24.0%	14.0%
5000	83.0%	43.0%	29.0%

[a] Egg mass density divided by NOAEL

[b] Based on dose-response model of Davidson et al. (2001) detailed in Section 4.3.2.

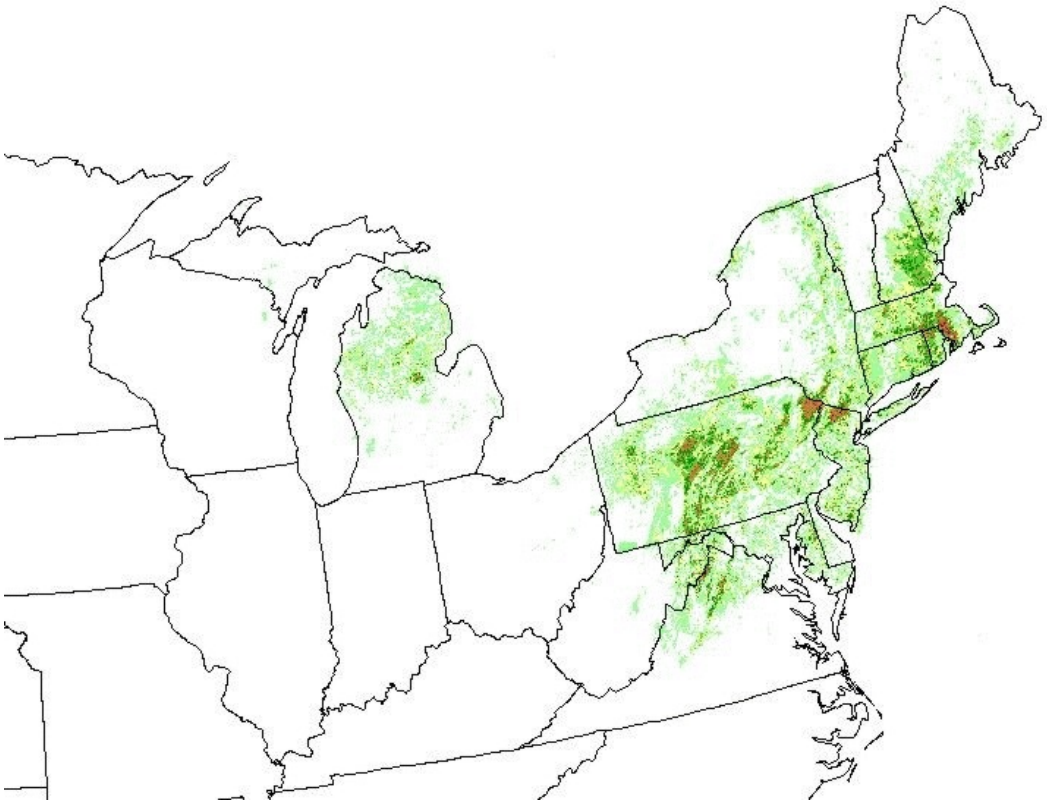

Figure 2-1: Frequency of defoliation by the gypsy moth from 1975 to 2002 (Source: http://www.fs.fed.us/ne/morgantown/4557/gmoth/defoliation/freq75_02.jpg)

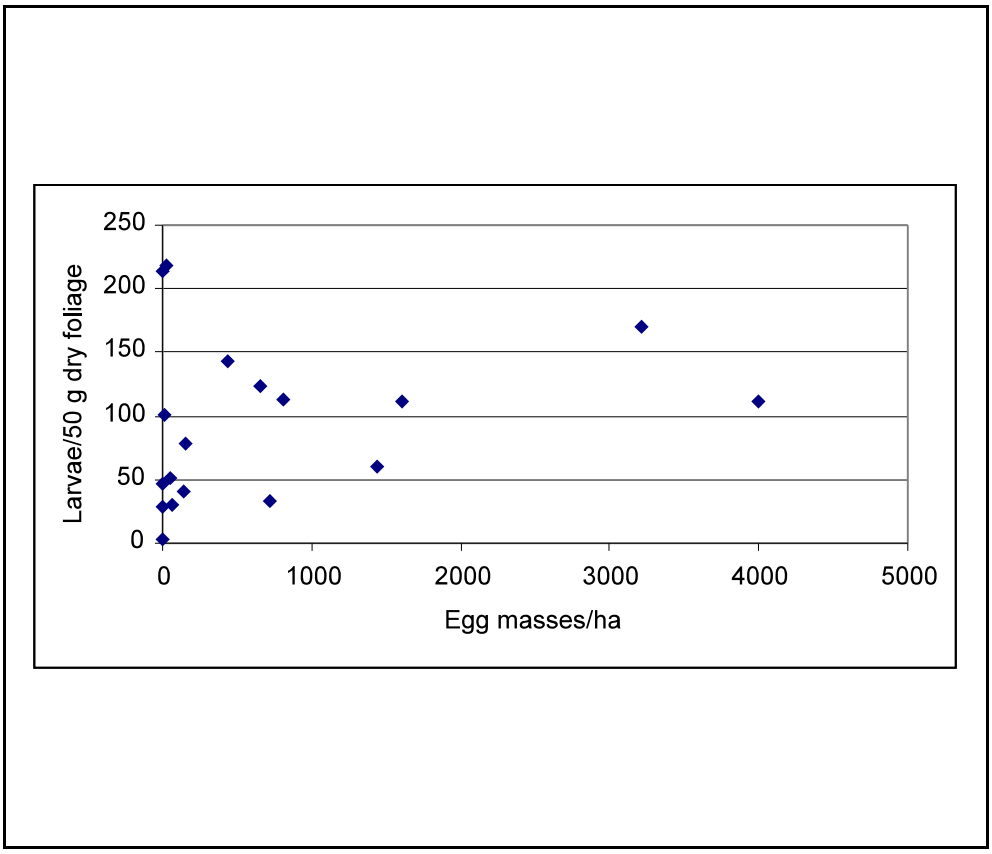

Figure 4-1: Relationship of egg mass density to number of larvae per 50 g dry weight of vegetation (Data from Sample et al. 1996).

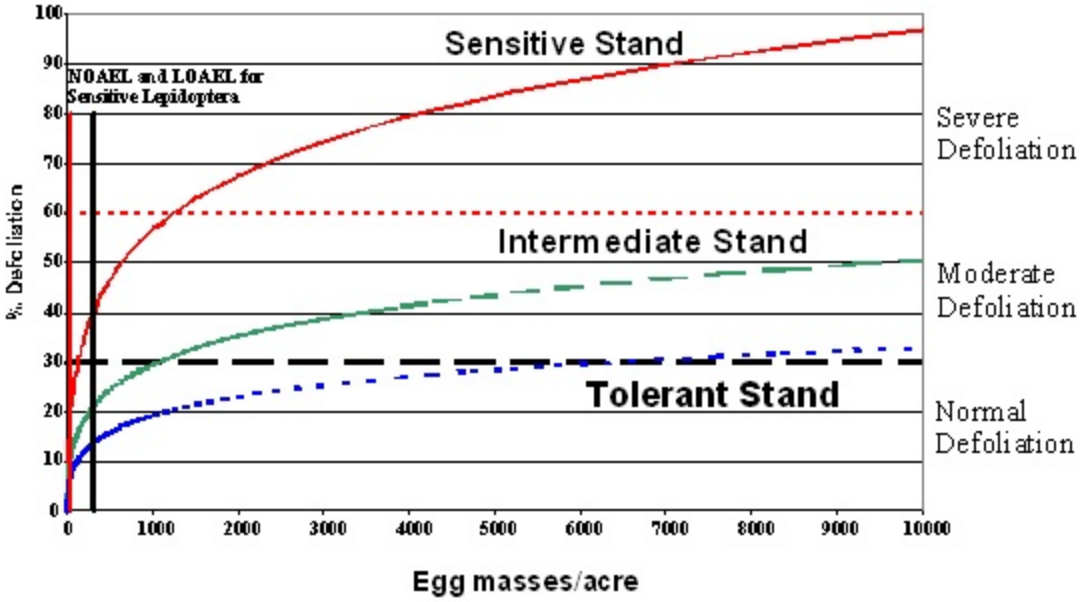

Figure 4-2: Summary of Exposure-Response Assessment (see text for details)

Appendix M
Risk Comparison

Figure M-1. Ropes were used to climb trees, to treat them for gypsy moths in the 1930s.

SERA TR 04-43-05-08e

Control/Eradication Agents for the Gypsy Moth - Risk Comparison – Final Report

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Figure 4-1: Risk comparison for potential effects in terrestrial species

Figure 4-2: Risk comparison for potential effects in aquatic species

NOTE: Tables and Figures are placed after Section 5, References.

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

a.i.	active ingredient
Ach	acetylcholine
AChE	acetylcholinesterase
AEL	adverse-effect level
APHIS	Animal and Plant Health Inspection Service
ARS	Agricultural Research Station
BCF	bioconcentration factor
B.t.k.	*Bacillus thuringiensis* var. *kurstaki*
BIU	Billions of international units
bw	body weight
4-CA	4-chloroaniline
ChE	pseudo-cholinesterase
CNS	central nervous system
DFB	diflubenzuron
EC_x	concentration causing X% inhibition of a process
EIS	environmental impact statement
FH	Forest Health
FS	Forest Service
FTU	forestry toxic units
HQ	hazard quotient
IRIS	Integrated Risk Information System
IU	international units
kg	kilogram
L	liter
LdNPV	gypsy moth (*Lymantria dispar*) nucleopolyhedrosis virus
lb	pound
LC_{50}	lethal concentration, 50% mortality
LD_{50}	lethal dose, 50% mortality
LD_{95}	lethal dose, 95% mortality
LOAEL	lowest-observed-adverse-effect level
m	meter
M	male
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
MSDS	material safety data sheet
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level

ACRONYMS, ABBREVIATIONS, AND SYMBOLS (*continued*)

NRC	National Research Council
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
OTS	Office of Toxic Substances
PIB	polyhedral inclusion body
ppm	parts per million
PVC	polyvinyl chloride
RfD	reference dose
RQ	risk quotients
UF	uncertainty factor
U.S.	United States
U.S. EPA	U.S. Environmental Protection Agency
USDA	United States Department of Agriculture

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

To convert ...	Into ...	Multiply by ...
acres	hectares (ha)	0.4047
acres	square meters (m^2)	4,047
atmospheres	millimeters of mercury	760
centigrade	Fahrenheit	1.8 °C + 32
centimeters	inches	0.3937
cubic meters (m^3)	liters (L)	1,000
Fahrenheit	centigrade	5/9 (°F-32)
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)	ug/square centimeter (ug/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
1×10^{-10}	0.0000000001	One in ten billion
1×10^{-9}	0.000000001	One in one billion
1×10^{-8}	0.00000001	One in one hundred million
1×10^{-7}	0.0000001	One in ten million
1×10^{-6}	0.000001	One in one million
1×10^{-5}	0.00001	One in one hundred thousand
1×10^{-4}	0.0001	One in ten thousand
1×10^{-3}	0.001	One in one thousand
1×10^{-2}	0.01	One in one hundred
1×10^{-1}	0.1	One in ten
1×10^{0}	1	One
1×10^{1}	10	Ten
1×10^{2}	100	One hundred
1×10^{3}	1,000	One thousand
1×10^{4}	10,000	Ten thousand
1×10^{5}	100,000	One hundred thousand
1×10^{6}	1,000,000	One million
1×10^{7}	10,000,000	Ten million
1×10^{8}	100,000,000	One hundred million
1×10^{9}	1,000,000,000	One billion
1×10^{10}	10,000,000,000	Ten billion

EXECUTIVE SUMMARY

OVERVIEW

The current document provides a comparison of the risks posed by the gypsy moth itself to the risks posed by the different control agents as well as a comparison of risks among the various control agents. The agents used in control programs include *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*), the gypsy moth nucleopolyhedrosis virus (LdNPV), diflubenzuron, tebufenozide, DDVP, and disparlure.

The gypsy moth itself poses the clearest risks in both the human health and ecological risk assessments. If the gypsy moth is not controlled, population outbreaks will occur and humans will be exposed to large numbers of gypsy moth larvae. If this occurs, a substantial number of individuals will experience skin irritation that is sufficiently severe to warrant medical attention. No more serious effects are likely. Ecologically, the gypsy moth will clearly damage some terrestrial vegetation and may directly affect some other species of moths. Because of the obvious importance of vegetation to the existence and habitat of most animals, defoliation by the gypsy moth will have numerous secondary effects.

Most of the control agents also pose risks and raise concerns, the nature and certainty of which are highly variable. In applications used to control the gypsy moth, *B.t.k.* is associated with irritant effects in humans; however, the severity of these effects appears to be less than those associated with exposure to the gypsy moth itself. The potential for *B.t.k.* to cause more serious human health effects is considered but appears to be remote. *B.t.k.* may also cause adverse effects in nontarget *Lepidoptera*. Concern for this effect is heightened because some of the *Lepidoptera* that may be adversely affected include at least on endangered species. Diflubenzuron does not appear to present any substantial risk to human health, and this assessment encompasses 4-chloroaniline, a potential carcinogen that is formed in the degradation of diflubenzuron. Diflubenzuron, however, is a rather nonspecific insecticide and is likely to impact both terrestrial and aquatic arthropods. Tebufenozide is a somewhat more specific insecticide but is used at higher application rates that may lead to high exposures in some terrestrial mammals. The likelihood of observing adverse effects, however, is unclear. Tebufenozide may also impact some nontarget moths and butterflies but should not adversely affect any aquatic species. Although DDVP is a broad spectrum insecticide and can be highly toxic to humans, adverse human health and ecological effects are not expected under normal conditions of use. If DDVP is improperly handled, exposures could substantially exceed prudent levels. For disparlure, exposure estimates for aquatic invertebrates approach a level of concern. More significantly, there is substantial uncertainty in the risk characterization of disparlure because of the limited acute toxicity data, the lack of chronic toxicity data, and the high likelihood that many species will be exposed to this compound.

Unlike all of the other agents considered in this risk assessment, there is no basis for asserting that the use of LdNPV to control or eradicate gypsy moth populations is likely to cause any adverse effects in any species other than the gypsy moth.

PROGRAM DESCRIPTION

The USDA control programs for the gypsy moth are intended to limit damage to forests that can be substantially impacted by gypsy moth outbreaks. Two biological agents that are pathogenic to the gypsy moth are used in broadcast applications: *B.t.k.* and LdNPV. In addition, three chemical agents are used in broadcast applications: diflubenzuron, tebufenozide, and disparlure. Diflubenzuron and tebufenozide are both insecticides, and, as discussed in subsequent sections of the this document, are quite similar with respect to their toxicological properties. The major difference between the two is that application rates for tebufenozide are higher than those for diflubenzuron and this is a controlling factor in the comparative risk assessment for these two agents. Disparlure is a gypsy moth pheromone that is used in broadcast applications to disrupt mating and in population monitoring programs to attract the male gypsy moth to sampling traps. In the past, disparlure was used in a slow-release flake formulation. DDVP is not used in broadcast applications and is used only as a PVC formulated product in milk carton traps used in mass trapping operations.

The USDA adopted various intervention strategies roughly categorized as suppression, eradication, and slow-the-spread. Suppression programs have relied predominantly on *B.t.k.* and diflubenzuron. Slow-the-Spread programs rely predominantly on the use of disparlure flakes and secondarily on *B.t.k.* applications. Eradication efforts rely predominantly on *B.t.k.* NPV is used in all three strategies but is used on very few acres relative to *B.t.k.*, diflubenzuron, and disparlure flakes.

HUMAN HEALTH RISK ASSESSMENT

Hazard Identification – The gypsy moth, *B.t.k.*, and LdNPV are similar not only because they are biological agents but also because the primary effect associated with each agent is irritation. The gypsy moth causes more pronounced and severe irritation relative to either *B.t.k.* or LdNPV. Of the chemical agents used in gypsy moth control programs, diflubenzuron and tebufenozide are similar to each other in that both cause adverse effects on blood. The risk assessment of diflubenzuron is somewhat more involved than that of tebufenozide because diflubenzuron is degraded to 4-chloroaniline, a compound that is classified as a carcinogen. DDVP and disparlure, the other two chemicals used in gypsy moth control programs, have toxicologic profiles that are very different from each other as well as diflubenzuron or tebufenozide. DDVP is a a well-characterized neurotoxin which was studied extensively in mammals. Disparlure, an insect attractant, was not tested extensively for toxicological effects in mammals.

Exposure Assessment – The exposure assessments of the biological agents differ substantially from those of the chemical agents in terms of how the exposures are expressed. Because of the available exposure and toxicity data, different measures of exposure are used for each of the biological agents – i.e., the gypsy moth, *B.t.k.*, and LdNPV. For the chemical agents, all exposure assessments are based on the amount or concentration of the chemical to which an individual or population might be exposed via ingestion, dermal contact, or inhalation. Differences among the chemical agents are dictated largely by differences in how the chemicals are used and, to a lesser extent, on the available toxicity data.

A very different set of exposure assessments is conducted for each of the biological agents. Both *B.t.k.* and LdNPV may also be applied in broadcast applications and the routes of plausible exposure are the same as those for the chemicals applied in broadcast applications – i.e., oral, dermal, and inhalation. For *B.t.k.*, however, the most directly relevant data used to characterize risk are based on actual applications of *B.t.k.* formulations where exposure is best characterized as an application rate. For the assessment of the potential for serious adverse effects, exposures are measured in colony forming units (cfu). LdNPV differs from all of the other agents in that no clear hazard potential can be identified; consequently, the most meaningful measure of exposure is, in some respects, moot. Those exposures that are quantified in the human health risk assessment for LdNPV are based on the mass of the formulation, Gypchek. Exposures to the gypsy moth itself are based on an indirect measure of exposure, egg masses/acre, because this is the expression of exposure that is used in the dose response assessment.

Differences in the exposure assessments among the chemicals used in USDA programs primarily reflect differences in how the chemicals are applied, what routes of exposure are most substantial, and the nature of the toxicity data. Diflubenzuron, tebufenozide, and disparlure may be applied in aerial broadcast applications and multiple routes of exposure (oral, dermal, and inhalation) are plausible. No chronic exposures for disparlure are conducted, however, because no chronic toxicity data are available on this chemical. DDVP, on the other hand, is used only in milk carton traps and exposures will be minimal under normal conditions, although much higher exposures are possible if the traps are not assembled properly or if individuals tamper with the traps.

Dose-Response Assessment – Dose-response assessments are typically based on an RfD, an estimate of a dose or exposure that is not likely to induce substantial adverse effects in humans. The RfD, in turn, is typically based on a NOAEL (no observed adverse effect level) divided by an uncertainty factor. Risk is then characterized as a hazard quotient (HQ), which is the estimated level of exposure divided by the RfD. If the HQ is below unity—i.e., the exposure is less than the RfD —there is no credible risk. If the HQ is above unity, risk is characterized based on dose-response or dose-severity relationships. The quality of the dose-response assessment depends on the quality of the individual studies, the relevance of the studies to potential human exposures, and the strength of the dose-response relationship.

As in the exposure assessments, the dose-response assessments for the biological agents differ substantially from each other as well as from those of the chemical agents. The dose-response assessment for the gypsy moth itself is based on only a single study; however, the study involves two human populations and demonstrates a clear dose-response relationship. Thus, confidence in the dose-response assessment is high. Two endpoints are considered for *B.t.k.*, irritant effects and more serious toxic effects. While the irritant effects are well documented, there is no apparent dose-response relationship and confidence in the dose-response assessment is classified as medium. The dose-response assessment for more serious effects is based on a single study on mice involving intratracheal exposures. While a clear dose-response relationship is apparent, confidence in the dose-response assessment is low because intratracheal exposures have marginal

(if any) relevance to human exposures, the response was not independently replicated, and the observed response might be an artifact. For LdNPV, no endpoint of concern can be identified. Although the individual studies conducted on LdNPV are somewhat dated, the weight of evidence for LdNPV as well as other similar viruses clearly indicates that no systemic effects in humans are anticipated. Thus, confidence in the dose-response assessment for LdNPV is classified as high.

Following standard practices in USDA risk assessments, risk assessment values available from U.S. EPA are adopted directly unless there is a compelling basis for doing otherwise. This approach is taken because the U.S. EPA will typically devote substantial resources and expertise to the development of risk assessment values and it is not feasible to duplicate this effort in risk assessments prepared for the USDA. In addition, the U.S. EPA has the legislative mandate to develop risk values for pesticides and it is sensible for the USDA to administratively defer to U.S. EPA in this area. When risk values are not available from U.S. EPA, the methods used by U.S. EPA are employed to derive surrogate values. Except for disparlure, chronic RfD values are available from U.S. EPA and these values are used directly. For 4-chloroaniline, the U.S. EPA also derived a cancer potency factor as well as a chronic RfD and these values are used directly in the risk assessment. For DDVP, the U.S. EPA derived an acute RfD, and this value is also adopted in the current risk assessment. A complication with DDVP, however, is that this agent is contained within a PVC strip, which substantially impacts the bioavailability of DDVP. In order to consider this detail quantitatively, a single and somewhat marginal study on the toxicity of DDVP in a PVC strip is used, and confidence in this dose-response assessment is, in turn, marginal. Unlike all of the other chemicals considered in this comparative risk assessment, very little toxicity data are available on disparlure. The U.S. EPA did not derive an RfD for this chemical, and the toxicity data available on disparlure are insufficient to derive a surrogate RfD. Thus, confidence in the dose-response assessment for disparlure is marginal.

Risk Characterization – Of the agents considered in this risk assessment, the gypsy moth and DDVP are clearly agents of marked concern, although the nature of the concerns is different. If the gypsy moth is not controlled, population outbreaks will occur and humans will be exposed to large numbers of gypsy moth larvae. If this occurs, a substantial number of individuals will experience irritant effects that are sufficiently severe to cause these individuals to seek medical attention. No more serious effects are likely. For DDVP, the potential for risk is clear but the likelihood of observing risk seems to be remote. Under normal conditions and proper handling, levels of exposure to DDVP will be negligible and risk will be inconsequential. Workers who mishandle a DDVP-PVC strip and/or members of the general public who handle a DDVP-PVC strip may be exposed to levels of DDVP that are far above acceptable levels. While such exposures are clearly to be avoided, they are not likely to cause frank signs of toxicity. This conclusion is consistent with human experience in the use of DDVP resin strips.

Diflubenzuron and tebufenozide are agents of marginal concern. Under most foreseeable conditions of exposure—i.e., exposure scenarios that might be characterized as typical—exposure levels will be far below levels of concern. At the upper ranges of plausible

exposure – levels that might be characterized as extreme— the hazard quotients for diflubenzuron approach a level of concern (HQs between 0.1 and 0.5 for both diflubenzuron and its 4-chloroaniline metabolite). For tebufenozide, the highest hazard quotient is 1.5, which is characterized as undesirable; however, exposure is not likely to cause overt signs of toxicity. The somewhat higher hazard quotients for tebufenozide, compared with those of diflubenzuron, are due solely to higher application rates.

Among the agents of minimal concern, *B.t.k.* is somewhat problematic. Based on the risk for serious adverse effects, there is clearly no cause for concern (the highest HQ is 0.04). As detailed in the dose-response assessment, this lack of concern is reenforced by a very aggressive and protective interpretation of the available toxicity data. Nonetheless, there is some residual concern with irritant effects. These effects are quite plausible in accidental cases of gross over-exposure – e.g., splashing a formulation into the eye. These kinds of concern are minimal and are common to almost all chemical or biological agents. The more troubling concern involves studies of workers and non-workers who report irritant effects, primarily throat irritation. Whether or not these effects should be attributed to the *B.t.k.* exposure is unclear.

The risk characterization for LdNPV and disparlure is unequivocal. Based on the available information, there is no basis for asserting that any serious adverse effects are plausible. Again, various accidental exposures, including splashing the agent into the eyes, could cause transient irritant effects.

ECOLOGICAL RISK ASSESSMENT

Hazard Identification – Unlike the human health risk assessment, in which the potential effects of the biological agents were similar, the ecological effects profile of each of the biological agents considered in this risk assessment are quite distinct. The gypsy moth will primarily affect sensitive trees, and these effects may be substantial. Because of the obvious importance of vegetation to the existence and habitat quality of most animals, a large number of secondary effects may be produced in many other groups of organisms. There is little indication, however, that the gypsy moth will have marked direct effects on groups of organisms other than sensitive plants. LdNPV, on the other hand, is not likely to have any effect on any species other than the gypsy moth. *B.t.k.* is toxic to nontarget *Lepidoptera* as well as the gypsy moth and some other lepidopteran species. There is very little indication that direct effects on other groups of organisms are plausible. Thus, the potential effects of all of the biological agents are considered relatively specific, with LdNPV showing the greatest degree of specificity (only the gypsy moth), followed by the gypsy moth itself (several types of plants) and *B.t.k.* (several types of *Lepidoptera*).

The chemical agents also differ in specificity: disparlure is most specific, tebufenozide is relatively specific to *Lepidoptera*, diflubenzuron is less specific and may affect many arthropods, and DDVP is a nonspecific biocide toxic to many groups of animals, especially arthropods and vertebrates. As a pheromone, disparlure is almost as specific as LdNPV. It will attract the gypsy moth and two other closely related species, the nun moth (*Lymantria monacha*) and the pink

gypsy moth (*Lymantria fumida*). As with the gypsy moth, both of these *Lymantria* species are forest pests, and adverse effects on these species are not a substantial concern for this risk assessment. In addition, the pink gypsy moth is native to Japan and is not found in the United States. A major qualification with the assessment of the specificity of disparlure is that, as in the human health risk assessment, the information on the toxicity of disparlure to nontarget species is very limited. At least in *Daphnia magna*, a commonly used test species in aquatic toxicity studies, the toxicity of disparlure is relatively high. Both diflubenzuron and tebufenozide are clearly toxic to mammals and at least some arthropods. In mammals, both chemicals will cause adverse effects in blood (methemoglobinemia), as detailed in the human health risk assessment. In both terrestrial and aquatic arthropods, both chemicals will interfere with growth and development. Because of differences in the mechanism of action of diflubenzuron and tebufenozide, tebufenozide appears to be somewhat more selective. Effects in birds have been clearly demonstrated for tebufenozide but not for diflubenzuron. While somewhat speculative, it seems plausible to assert that both diflubenzuron and tebufenozide are likely to affect the blood of birds in a way similar to that seen in mammals. In terms of the mechanism of action, DDVP is a general neurotoxin. In all animals that have nervous systems that involve acetylcholinesterase (AChE) and use acetylcholine (ACh) as a neurotransmitter (a substance necessary to make the nerves work properly), DDVP will be toxic, and sufficiently high exposures to DDVP will be lethal. The definition of *sufficiently high*, however, is critical and variable. Although DDVP is not selective mechanistically, differences in sensitivity among species are substantial. For instance, insects are much more sensitive than mammals or other higher organisms to DDVP exposure. This difference in sensitivity is what characterizes DDVP as an effective insecticide that can be used safely.

Exposure Assessment – Diflubenzuron, tebufenozide, LdNPV, and disparlure may be applied in broadcast applications, which means that the potential for exposure is high and, in many cases, unavoidable. Disparlure, in addition to being used in broadcast applications, is used in traps as an attractant. Under those conditions of use, exposure to disparlure will be variable and primarily incidental. Exposures to the gypsy moth itself also vary, depending on the state of the gypsy moth population—i.e., from low level infestation to outbreak conditions.

Some differences between the human health exposure assessment and the ecological exposure assessment, however, are notable. Table 4-2 does not give a measure of exposure for each agent. This is because the measure of exposure will vary both among agents and among the target groups for each agent. For example, exposures to the gypsy moth are measured as egg masses/acre in the human health risk assessment and this is the same measure of exposure used for terrestrial vegetation. As in the human health risk assessment, egg masses/acre are used as the measure of exposure because this is the primary determinant in the dose-response assessment for plants. For all other species, however, effects from the gypsy moth are likely to be secondary rather than primary. Thus, the exposure assessment for these indirectly affected species is based on defoliation – i.e., the result of the dose-response assessment for terrestrial vegetation is used as the exposure assessment for most other groups of organisms.

Other differences in the exposure assessments for nontarget species are mostly superficial. For each of the chemical agents, the mass of the chemical is typically used as the measure of exposure. Depending on the group, the measure of exposure may be expressed as dose (mg agent/kg bw for most terrestrial species), concentration (mg agent/L of water for aquatic species), or simply as application rate (lb agent/acre). This last measure is used primarily when field studies are the basis for the dose-response assessment.

As in the human health risk assessment, different measures of exposure are used for each of the biological agents. For *B.t.k.*, most of the exposures are characterized simply as an application rate in units of BIU/acre. However, colony forming units are used for some of the mammalian exposure scenarios. Also as in the human health risk assessment, no clear hazard potential is identified for LdNPV. The very few exposure scenarios that are quantified in the ecological risk assessment for LdNPV are based on the mass of the formulation, Gypchek.

The level of detail used in the exposure assessments for the different chemicals reflects both differences in use patterns and the nature of the available toxicity data. Full sets of exposure assessments in several groups of animals are developed for diflubenzuron and tebufenozide. As in the human health risk assessment, the exposure assessment for diflubenzuron is elaborated by the consideration of 4-chloroaniline and the exposure assessment for tebufenozide is elaborated by the consideration of multiple applications.

Disparlure, which also may be applied in aerial broadcast applications, has a much more restricted set of exposure scenarios on far fewer groups of organisms. This difference is due completely to the sparse toxicity data available on this compound. In other words, while a very elaborate set of exposure scenarios could be prepared, these scenarios would serve little purpose because they could not be combined with a dose-response assessment to characterize risk. The exposure assessment for DDVP is also restricted but this restriction is due to the very limited exposures that are plausible because DDVP is used only in milk carton traps and exposures for nontarget species will be minimal under normal conditions.

Dose-Response Assessment – In general, confidence in any dose-response relationship is enhanced if a clear dose-response relationship can be demonstrated and both effect and no-effect exposures have been identified. In the case of LdNPV, however, there is simply no indication that LdNPV or the Gypchek formulation will cause toxicity in any nontarget species at any dose level. All of the risk values for LdNPV are based on no-effect concentrations or doses. While additional studies could be conducted at higher doses and while these studies would enhance confidence in the risk assessment, the NOAEL and NOEC values that have been identified are far above any plausible exposures. Thus, while based on limited data in terms of dose-effect characterization, the dose-response assessment for LdNPV is adequate for risk characterization.

For most of the other agents, the dose-response assessments are reasonably good for the species of greatest concern. Dose-response assessments for DDVP are derived only for mammals, fish, and aquatic invertebrates. This limited approach is taken with DDVP because of the limited use

of DDVP in programs to control the gypsy moth. The DDVP is contained in a PVC strip that is placed in a milk carton trap that includes disparlure as an attractant for the gypsy moth. This type of use limits potential exposure to most nontarget species. A formal dose-response assessment is not conducted for terrestrial invertebrates. This is not due to any lack of data. The toxicity of DDVP to insects and many other invertebrates is very well characterized. DDVP is such a potent insecticide that no formal dose-response assessment is needed. Insects and many other species that enter the trap are likely to be killed by exposure to DDVP.

Disparlure is the other agent for which a full set of dose-response assessments is not developed. As discussed in the hazard identification, this is due to the very limited data that are available on the toxicity of disparlure to nontarget species.

Relatively full dose-response assessments on groups of greatest concern are given for the gypsy moth, *B.t.k.*, diflubenzuron and its 4-chloroaniline metabolite, and tebufenozide. For the gypsy moth, the effect of primary concern is damage to vegetation. While data are available on both lethality in trees as well as defoliation, defoliation is used as the sublethal effect of primary concern. A dose-response assessment is also given for nontarget lepidopterans. While effect and no-effect levels can be identified, the significance of this effect is questionable. In terms of direct effects, terrestrial vegetation is the primary target of concern.

The primary nontarget group of concern for *B.t.k.* involves *Lepidoptera*. A relatively rich set of studies is available in which the sensitivities of nontarget *Lepidoptera* as well as some other insects can be quantified reasonably well based on studies involving exposures that encompass the application rates used to control the gypsy moth. Sensitive nontarget lepidoptera include larvae of the endangered Karner blue butterfly as well as several other types of moths.

Similar types of information are available on diflubenzuron and tebufenozide, and dose-response assessments can be made for the species of primary concern. For both chemicals, this includes nontarget *Lepidoptera* and aquatic invertebrates. Other terrestrial arthropods are also considered for diflubenzuron. In addition, because of the standard tests required by U.S. EPA for the registration of most pesticides, adequate toxicity data are available on mammals, birds, and fish. The toxicity data base for diflubenzuron is somewhat more extensive and sensitivities in nontarget organisms are somewhat better defined in both laboratory and field studies than is the case with tebufenozide.

Risk Characterization – Ecological risk assessments involve, at least implicitly, considerations of thousands of different species and relationships among these species and their habitats. Invariably, however, data are available on only a small subset of these species and field studies provide only limited insight into the complex interrelationships and secondary effects among species. Thus, as in the human health risk assessments, ecological risk assessments cannot offer a guarantee of safety. They can and do offer a means to identify whether or not there is a basis for asserting that adverse effects are plausible and what the nature of these effects might be.

Within these limitations, only LdNPV clearly qualifies as an agent of minimal concern. While there are limitations in the available studies on LdNPV, there is simply no basis for asserting that LdNPV will adversely affect any species except the gypsy moth.

Agents of marked concern included the gypsy moth, *B.t.k.*, and diflubenzuron. The types of concern with each of these agents, however, are quite different. For both the gypsy moth and *B.t.k.*, the concerns are narrow. The gypsy moth will clearly damage some terrestrial vegetation. *B.t.k.* is likely to affect sensitive *Lepidoptera*. Concern with the use of diflubenzuron is broader and includes effects on both terrestrial and aquatic invertebrates.

The designation of the gypsy moth as an agent of marked concern is obvious. The effects of gypsy moth larvae on forests are extremely well documented and well understood. In sensitive forest stands—i.e., stands in which oak, birch, and other favored species predominate —gypsy moth larvae can cause substantial defoliation and tree mortality. While some other lepidopteran species also may be directly affected by exposure to the gypsy moth, most of the other effects caused by the gypsy moth will be secondary. Reductions in populations of squirrels, mice, and other mammals which may be sensitive to changes in the availability of acorns are likely and have been well documented. Substantial secondary adverse effects on other groups of animals – i.e., birds, reptiles, and aquatic species – cannot be ruled out but have not been convincingly or consistently demonstrated.

Diflubenzuron is also clearly an agent of marked concern. Exposures to diflubenzuron at application rates used in gypsy moth control programs will adversely affect both terrestrial and aquatic invertebrates that rely on chitin for their exoskeleton. This effect is demonstrated in controlled toxicity studies as well as multiple field studies.

B.t.k. is considered an agent of marked concern because recent studies convincingly demonstrate that adverse effects in nontarget *Lepidoptera* will occur in the applications of *B.t.k.* used to control the gypsy moth. Concern is heightened because some of the *Lepidoptera* that may be adversely affected include at least one endangered species.

Tebufenozide, DDVP, and disparlure are all classified as agents of marginal concern. For tebufenozide, the numeric expressions of risk may be less relevant than a more qualitative assessment. The highest risk is associated with the consumption of contaminated vegetation by a large mammal after two applications at the highest labeled application rate. It is not clear, however, that any frank signs of toxicity would be seen. Risks to nontarget *Lepidoptera* may be of greater concern, but the available data are insufficient to quantify potential risk. Risks to other invertebrates, both terrestrial and aquatic, appear to be insubstantial. DDVP is of marginal concern in that highly localized effects may be expected: nontarget insects entering a milk carton trap or some aquatic invertebrates affected by the accidental contamination of a small body of water with a pest strip. In both cases, the effects would be relatively minor, in terms of the number of organisms affected. Marginal concern for disparlure is associated with the relatively high toxicity of this agent to *Daphnia*. The very limited information on the toxicity of disparlure

argues for a persistent level of vigilance for this agent that may be applied to large areas in broadcast applications.

1. INTRODUCTION

The USDA is preparing an update to the 1995 Environmental Impact Statement (EIS) for the Cooperative Gypsy Moth Management Program (USDA 1995). As part of this effort, updated risk assessments were developed on each of the chemical and biological control agents that are used in the USDA programs:—i.e., *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*) (SERA 2004a), the gypsy moth nucleopolyhedrosis virus (LdNPV) (SERA 2004b), diflubenzuron (SERA 2004c), tebufenozide (SERA 2004f), DDVP (SERA 2004e), and disparlure as an active ingredient in materials used to attract the gypsy moth (SERA 2004d). In addition, a separate risk assessment was prepared on the gypsy moth (*Lymantria dispar*) itself.

The current document not only compares the risks posed by the gypsy moth itself with the risks posed by the different control agents, but also compares the risks associated with the various control agents. The risk comparison is structured like the individual risk assessments and includes comparisons of uses (Section 2), potential human health effects (Section 3), and potential ecological effects (Section 4). As in the individual risk assessments, each of the comparative risk assessment sections (Sections 3 and 4) has four major subsections, including an identification of the hazards associated with the agents, an assessment of potential exposure, an assessment of the dose-response relationships, and a characterization of the risks associated with each agent.

Each of the individual risk assessments cited above are complex, detailed, and often very large documents. The risk comparison does not attempt to summarize this information again in detail. Instead, it focuses on discussing the nature and quality of the data that support each step of the risk assessments and the uncertainties and limitations in the conclusions that are reached. Thus, with few exceptions, individual studies are not discussed or referenced in the current document. The exceptions primarily involve relatively recent studies that substantially impact the assessment of risk. Most of these studies involve *B.t.k.* (Herms et al. 1995; Hernandez et al. 1999, 2000; Peacock et al. 1998; Petrie et al. 2003).

2. PROGRAM DESCRIPTION

2.1. Overview

The USDA control programs for the gypsy moth are intended to limit damage to forests that can be substantially impacted by gypsy moth outbreaks. Two biological agents that are pathogenic to the gypsy moth are used in broadcast applications: *B.t.k.* and LdNPV. In addition, three chemical agents are used in broadcast applications: diflubenzuron, tebufenozide, and disparlure. Diflubenzuron and tebufenozide are both insecticides and, as discussed in subsequent sections of the this document, have similar toxicologicalal properties. The major difference between the two is that application rates for tebufenozide are higher than those for diflubenzuron, which is a controlling factor in the comparative risk assessment for these two agents. Disparlure is a gypsy moth pheromone used in broadcast applications to disrupt mating and in population monitoring programs to attract the male gypsy moth to sampling traps. In the past, disparlure was used in a slow-release flake formulation. DDVP is not used in broadcast applications and is used only as a PVC formulated product in milk carton traps used in mass trapping operations.

The USDA adopted various intervention strategies roughly categorized as suppression, eradication, and slow-the-spread. Suppression programs have relied predominantly on *B.t.k.* and diflubenzuron. Slow-the-Spread programs rely predominantly on the use of disparlure flakes and secondarily on *B.t.k.* applications. Eradication efforts rely predominantly on *B.t.k.* NPV is used in all three strategies but is used on very few acres relative to *B.t.k.*, diflubenzuron, and disparlure flakes.

2.2. Control Agents

Gypsy moth is a pest species that can cause substantial damage to some forests. In the eastern United States, most hardwood forests are classified as susceptible to gypsy moth infestation and as many as 12.5 million acres have been defoliated in a single season. The gypsy moth is found throughout much of New England and south to Virginia and west to portions of Wisconsin.

In past years, USDA employed chemical and biological agents in gypsy moth control programs. The biological control agents consist of *B.t.k.* and LdNPV. Both of these biological agents are pathogenic to the gypsy moth. The chemicals that may be used in the control of the gypsy moth include diflubenzuron, tebufenozide, and disparlure. Diflubenzuron and tebufenozide are used as direct insecticidal control agents, similar to the uses of *B.t.k.* and LdNPV. All of these agents are used in broadcast aerial or ground applications.

DDVP and disparlure are used in mass trapping. Disparlure attracts the male gypsy moth to a large milk carton trap and the DDVP kills insects that enter the trap. While DDVP functions as an insecticide in the trap, it is not considered a control agent for the gypsy moth because mass trapping is used only in population surveys. Disparlure, in a flake formulation, is also used in broadcast aerial applications. While the disparlure does not cause any direct toxic effects to the gypsy moth, the mass application of disparlure will impair the ability of the male gypsy moth to

find female gypsy moths and thus will limit the ability of gypsy moth populations to propagate. Thus, disparlure is used as a control agent.

All of the agents used in gypsy moth control programs are applied in various types of formulations—i.e., the active ingredient combined with various other chemicals or materials. To the extent possible, these materials are discussed in each of the individual risk assessments. Specific information on inerts, however, is classified as CBI (confidential business Information) under Section 7(d) and Section (10) of FIFRA, and this information cannot be specifically disclosed in a risk assessment. In terms of a comparative risk assessment, however, the most important distinctions involve the formulations of *B.t.k.* and LdNPV in complex mixtures and the use of DDVP in polyvinyl chloride (PVC) strips.

B.t.k. and LdNPV are both applied as very complex mixtures that are not fully or clearly defined. *B.t.k.* is cultured or grown in a medium containing water and nutrients, including sugars, starches, proteins, and amino acids. The nutrients, which are, themselves, chemically complex consist of variable biological materials, including animal foodstuffs, various flours, yeasts, and molasses. Similarly, LdNPV is prepared by isolating the virus from infected gypsy moth larvae. The active material consists of the virus, gypsy moth parts, and residual materials used to isolate and purify the virus. Complex mixtures can pose substantial difficulties in a risk assessment; however, the data on *B.t.k.* and LdNPV involve adequate studies on the toxicity of the complex mixtures. This is particularly true for *B.t.k.* in which much of the information on risk is based on applications of commercial formulations in the field.

DDVP is used only in a PVC strip. Each strip contains 590 mg of DDVP and 89.25% inerts, which consist primarily of the PVC in the strip and plasticizers. The limited use of DDVP and its containment in the PVC strip have a major impact on the risk posed by DDVP, relative to the other compounds used in gypsy moth control programs. This impact is discussed at some length in the DDVP risk assessment and in subsequent sections of this document.

2.3. Application Rates

Application rates for the different control agents differ substantially both in magnitude and, for the biological agents, in the manner in which the application rate is expressed.

For *B.t.k.*, application rates are expressed in billions of international units (BIU), which is a measure of the activity or potency of the formulation rather than an expression of mass. The range of application rates used in USDA programs is 20-40 BIU/acre. For LdNPV, the recommended application rate is 0.43 oz Gypchek/acre for suppression and 1.08 oz Gypchek/acre for eradication. The application rate of 0.43 oz/acre corresponds to about $4x10^{11}$ PIB (polyhedral inclusion bodies)/acre and the application rate of 1.08 oz/acre corresponds to about $1x10^{12}$ PIB/acre.

Broadcast application rates are expressed in units of lb a.i./acre. For diflubenzuron, the range of labeled application rates is 0.0078-0.0624 lbs a.i./acre. For tebufenozide, higher labeled

application rates are permitted: 0.03-0.12 lbs/acre. Multiple applications of tebufenozide are also permitted, and the maximum annual application rate is 0.24 lb a.i./acre. The application rates for tebufenozide may vary among USDA programs—i.e., suppression, eradication, and slow-the-spread. For the tebufenozide risk assessment, a range of application rates—i.e., 0.015- 0.12 lb a.i./acre—are considered. All exposure assessments are conducted at the maximum application rate of 0.12 lb/acre, assuming that two applications are made with three days between applications. This worse-case scenario involves the use of two applications that reach the maximum annual application rate of 0.24 lb/acre and the shortest interval between applications. As noted in Section 3.4, the higher application rates for tebufenozide, compared with application rates for diflubenzuron, are the determining factor in the risk comparison. The application rate for disparlure is about 0.064 lb a.i./acre, near the maximum application rate allowed for diflubenzuron. Disparlure, however, is always applied in a slow-release formulation, either flakes or microspheres. DDVP is not applied in broadcast applications. Accordingly, the application rate is not a meaningful measure of exposure for this agent.

2.4. Use Statistics

In order to minimize the ecological effects and human health effects of gypsy moth infestations, the USDA adopted various intervention strategies roughly categorized as suppression, eradication, and slow-the-spread (Liebhold and McManus 1999). Suppression efforts are conducted by the USDA Forest Service in areas of well established gypsy moth infestations to combat or interdict periodic gypsy moth population outbreaks. Eradication efforts are conducted by USDA/APHIS to eliminate gypsy moth populations in areas where new populations of the gypsy moth are found. Slow-the-Spread, as the name implies, is a program to reduce the expansion of gypsy moth populations from areas of established populations to adjacent non-infested areas.

The use of the various control agents in USDA programs is summarized in Table 2-1. This table gives the total number of acres treated with each of the control agents between 1995 and 2003. Suppression programs rely predominantly on *B.t.k.* and diflubenzuron. Slow-the-Spread programs rely predominantly on the use of disparlure flakes and secondarily on *B.t.k.* applications. Eradication efforts rely predominantly on *B.t.k.* NPV is used in all three strategies but is used on very few acres relative to *B.t.k.*, diflubenzuron, and disparlure flakes. As discussed in the risk assessment on NPV, the production of Gypchek is very expensive and the application of this agent is currently limited to areas that are considered environmentally sensitive. As noted above, tebufenozide is not used in gypsy moth programs but may be used in the future. Given the similarities between tebufenozide and diflubenzuron, the use of tebufenozide is likely to be similar to that of diflubenzuron—i.e., primarily in suppression programs.

3. HUMAN HEALTH RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION

3.1.1. Overview

An overview of the comparative hazard identification for the gypsy moth and the agents used in USDA programs to control the gypsy moth is given in Table 3-1. The gypsy moth, *B.t.k.*, and LdNPV are similar not only because they are biological agents but also because the primary effect associated with each agent is irritation. The gypsy moth causes more pronounced and severe irritation relative to either *B.t.k.* or LdNPV. Of the chemical agents used in gypsy moth control programs, diflubenzuron and tebufenozide are similar to each other in that both cause adverse effects on blood. The risk assessment of diflubenzuron is somewhat more involved than that of tebufenozide because diflubenzuron is degraded to 4-chloroaniline, a compound that is classified as a carcinogen. DDVP and disparlure, the other two chemicals used in gypsy moth control programs, have toxicological profiles that are very different from each other as well as from diflubenzuron or tebufenozide. DDVP is a well-characterized neurotoxin and the toxicity of DDVP in mammals has been studied extensively. Disparlure is an insect attractant that has not been extensively tested for toxicological effects in mammals.

3.1.2. Biological Agents

The biological agents—i.e., *B.t.k.*, LdNPV, and the gypsy moth itself—present similar toxicological profiles. All three agents are irritants and cause similar irritant effects. The most likely effect from exposure to the gypsy moth is skin irritation. Gypsy moth larvae, as well as the larvae of many species of *Lepidoptera*, cause skin irritation in humans. The skin reactions seem to be associated with contact with small fine hairs that stick out from the body of the larva. Other effects associated with exposure to gypsy moth larvae include eye and respiratory irritation; however, these effects are not as well documented as the dermal effects.

LdNPV also causes irritant effects. It is likely that the irritant effects are due at least in part to the presence of body parts of gypsy moth larvae in LdNPV preparations. Based on the available animal data, there is clear evidence that Gypchek, the commercial formulation of LdNPV, can cause eye irritation. There is little indication, however, that Gypchek is likely to cause dermal or respiratory irritation, which may have something to do with the processing of the gypsy moth parts during the preparation of Gypchek.

The irritant effects of *B.t.k.* are probably due to the formulation of the bacteria rather than the bacteria itself. As noted in Section 2, commercial preparations of *B.t.k.* are very complex mixtures of the bacteria, fermentation byproducts, and adjuvants. *B.t.k.* formulations, however, are not strong irritants to either the eyes or the skin, except in the cases of accidental and gross contamination of the eyes. Instead, the most consistent effect appears to be irritation of the respiratory tract, particularly the throat.

The irritant effects of the gypsy moth appear to be notably more severe than those of *B.t.k.* The wheals and rashes that result from exposure to the gypsy can cause severe itching which may

persist from several days to two weeks. Moreover, these effects can be severe enough to cause the affected individual to seek medical treatment. The relatively consistent set of epidemiology studies following *B.t.k.* applications note a very different outcome. Despite many reports of irritant effects among exposed individuals, there is not a corresponding increase in the incidence of individuals seeking medical care. Thus, unlike the case in severe gypsy moth infestations, the severity of the irritant effects does not appear to be severe enough for individuals to seek medical care.

There is very little indication that these biological agents will be associated with other more serious effects. LdNPV and Gypchek formulations of LdNPV were tested in relatively standard toxicity studies as well as in pathogenicity studies with no indication of serious effects even at very high doses. The gypsy moth has not been formally tested in human or animal studies; on the other hand, this species has infested North America for more than 100 years and no cases of frank adverse effects associated with gypsy moth exposure are to be found in the available literature. Hence, there appears to be no risk of serious adverse effects from exposure to LdNPV, Gypchek, or the gypsy moth itself.

The potential for *B.t.k.* to produce serious adverse effects is somewhat more complicated than the assessment of LdNPV and the gypsy moth. As discussed in the *B.t.k.* risk assessment, severe adverse effects associated with exposure to *B.t.k.* are not reported in any of several epidemiology studies or standard animal toxicity studies on *B.t.k.* or formulations of *B.t.k.* A recent study by Hernandez et al. (2000), however, reports mortality in mice after intranasal instillations of *B.t.k.* Intranasal instillations of bacteria are analogous to inhalation exposures in that the bacteria are inhaled and transported to the lungs during the course of the study. This route of exposure is used to screen qualitatively for potential toxic effects, particularly for biological agents, and is not commonly used in a quantitative risk assessment because of uncertainties in extrapolating from intranasal doses to inhalation exposures that may occur in humans. In the *B.t.k.* risk assessment, some very conservative assumptions are made in the application of the Hernandez et al. (2000) study to provide an estimate of risk. As with LdNPV and the gypsy moth, this analysis (considered further in Sections 3.3 and 3.4) suggests that the risk of adverse effects is likely to be very low under foreseeable conditions of exposure.

The Hernandez et al. (2000) study also reports that pre-treatment of mice with an influenza virus substantially increased mortality in mice exposed to various doses of *B.t.k.*, again by intranasal instillation. This effect raises concern about the susceptibility of individuals who have influenza or other viral respiratory infections to severe adverse responses to *B.t.k.* exposure. The viral enhancement of bacterial infections is not uncommon and the enhancement of *B.t.k.* toxicity by a viral infection is, in some respects, not surprising. The relevance of this observation to public health cannot be assessed well at this time. No such effects are reported in the epidemiology studies conducted to date. It is, however, not clear that the epidemiology studies would detect such an effect or that such an effect is plausible under the anticipated exposure levels (typical or extreme) used in programs to control the gypsy moth. The viral enhancement of *B.t.k.* toxicity is likely to be an area of further study in the coming years.

3.1.3. Chemical Agents

In terms of potential human health effects, diflubenzuron and tebufenozide are similar to one another in that both cause adverse effects on blood. DDVP and disparlure, the other two chemicals used in gypsy moth control programs, have toxicological profiles that are very different from one another as well as from diflubenzuron or tebufenozide. The toxicity of DDVP, which is a well-characterized neurotoxin, has been studied extensively in mammals. Disparlure is an insect attractant that has not been extensively tested for toxicologicalal effects in mammals.

3.1.3.1. Diflubenzuron and Tebufenozide – For both diflubenzuron and tebufenozide, the most sensitive effect in mammals involves damage to hemoglobin, a component in red blood cells that is responsible for transporting oxygen throughout the body. If this function is impaired, either because of damage to hemoglobin or lack of oxygen in the air, serious adverse effects (i.e., equivalent to suffocation) can occur. Both diflubenzuron and tebufenozide cause the formation of methemoglobin, a form of hemoglobin that is not able to transport oxygen. Both chemicals causes other effects on the blood; however, methemoglobinemia is the most sensitive effect—that is, the effect that occurs at the lowest dose.

While effects on the blood are well documented, there is less of an indication that diflubenzuron or tebufenozide will cause other specific forms of toxicity. Neither diflubenzuron nor tebufenozide appears to be carcinogenic, mutagenic, neurotoxic or immunotoxic. Furthermore, these chemicals do not appear to cause birth defects or affect endocrine function in laboratory mammals. Diflubenzuron does not appear to cause reproductive effects. Tebufenozide, on the other hand, is associated with adverse reproductive effects in experimental mammals. These reproductive effects, however, occur at doses higher than those associated with methemoglobinemia. Neither diflubenzuron nor tebufenozide have a high order of acute oral toxicity. Diflubenzuron is relatively nontoxic by oral administration, with reported single-dose LD_{50} values ranging from greater than 4640 to greater than10,000 mg/kg. Similarly, single oral gavage doses of tebufenozide at 2000 mg/kg caused no observable signs of toxicity in mice or rats.

Diflubenzuron is degraded to 4-chloroaniline in the environment. While most chemicals are metabolized in some way, the formation of 4-chloroaniline from diflubenzuron must be and is explicitly considered in the risk assessment because 4-chloroaniline is classified as a carcinogen. This is the only identified carcinogen associated with any of the agents used to control the gypsy moth.

3.1.3.2. DDVP – DDVP is an organophosphorus insecticide that works by inhibiting cholinesterase. Inhibition of this enzyme in mammalian systems produces a variety of systemic effects, including salivation, urination, lacrimation, convulsions, increased bronchial secretions, respiratory depression, and even death. The nature and magnitude of the toxic effects produced by a given exposure to DDVP by any route are directly related to the dose and rate at which the exposure occurs.

In the case of the USDA programs for the management of the gypsy moth, the use of milk carton traps containing Vaportape II (slow-release of DDVP from PVC strips) precludes rapid exposures to high doses of DDVP. The decrease in toxicity of DDVP in a PVC formulation has been studied directly. For the technical grade liquid DDVP, the acute oral LD_{50} in young pigs is about 160 mg/kg and signs of toxicity in these animals were consistent with the general signs of acetylcholinesterase (AChE) inhibition. In a similar bioassay using a PVC formulation, no deaths occurred at doses up to 1000 mg/kg. This key study on the comparative toxicity of DDVP and DDVP-PVC formulations is discussed further in the dose-response assessment (Section 3.3).

DDVP is a very well studied compound and threshold doses for cholinesterase inhibition are well characterized. Short-term animal studies using technical grade DDVP indicate that oral exposures to doses below about 0.5 mg/kg-day (or inhalation exposures to 1-2 mg/m^3) do not result in meaningful reductions in cholinesterase activity. Experiments in laboratory mammals exposed to DDVP during pregnancy (by oral or inhalation route) did not show any effect on fertility or health of the offspring, even at levels that produced maternal toxicity. The latest evaluation of data from assays for carcinogenicity and genetic toxicity classify DDVP as a "suggestive" carcinogen and determined that a quantitative assessment of cancer risk is not applicable. The literature contains some data suggesting that contact dermatitis (as well as cross-sensitization to other pesticides) may occur; although, this appears to be an infrequent occurrence in the general population.

3.1.3.3. Disparlure – In the registration of most pesticides, the U.S. EPA requires a relatively standard set of toxicity data covering multiple routes and durations of exposure as well as a number of specific endpoints of concern (e.g., carcinogenicity, reproductive toxicity, neurotoxicity, etc.). These requirements have been applied to diflubenzuron, tebufenozide, and DDVP but not to disparlure. Because of the apparently low toxicity of most pheromones to mammals and because of the low concentrations that are expected in the environment, U.S. EPA requires less rigorous testing of insect pheromones than is required of insecticides (U.S. EPA 2004).

The prudence of these assumptions may be argued but this issue is beyond the scope of the current risk assessment except to note that the application rate for disparlure is somewhat higher than the application rate for diflubenzuron—i.e., up to 0.0624 lbs a.i./acre for diflubenzuron and about 0.064 lb a.i./acre for disparlure (see Section 2). Nonetheless, as noted in Section 2, disparlure is always applied in a slow-release formulation (either flakes or microspheres) and the limited available monitoring data (Section 3.2), do support the assumption that exposures to disparlure are likely to be very low.

In terms of the hazard identification, the result of the U.S. EPA position and the more general lack of concern with the toxicity of insect pheromones is that the toxicity of disparlure to mammals has not been studied extensively. Except for some standard acute toxicity studies in laboratory mammals, few data are available regarding the biological activity of disparlure in mammals. Results of acute exposure studies for oral, dermal, ocular, and inhalation exposure to

disparlure show no indication of adverse effects. The acute toxicity of disparlure in mammals is very low. The LD_{50} of a single dose administered to rats by gavage exceeds 34,600 mg/kg. No studies investigating the effects of chronic exposure of mammals to disparlure or studies investigating the effects of disparlure on the nervous system, immune system, reproductive system, or endocrine system were identified. The carcinogenic potential of disparlure has not been assessed. In a single study on mutagenicity, there was no indication that disparlure is mutagenic.

A case report of an accidental exposure indicates that disparlure may persist in humans for years. This case report concerns an individual involved in the early testing of disparlure who came into contact with the chemical. For more than 10 years after exposure to disparlure, the individual tended to attract male gypsy moths. This nuisance effect is the only well documented result of exposures to disparlure that might occur in USDA programs.

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview

A summary of the exposure assessments for each of the agents covered in the risk assessment is given in Table 3-2. The exposure assessments of the biological agents differ substantially from those of the chemical agents in terms of how the exposures are expressed. Different measures of exposure are used for each of the biological agents—i.e., the gypsy moth, *B.t.k.*, and LdNPV. For the chemical agents, all exposure assessments are based on the amount or concentration of the chemical to which an individual or population might be exposed via ingestion, dermal contact, or inhalation. Differences among the chemical agents are dictated largely by differences in how the chemicals are used and, to a lesser extent, on the available toxicity data.

A very different set of exposure assessments is conducted for each of the biological agents. Both *B.t.k.* and LdNPV may also be applied in broadcast applications and the routes of plausible exposure are the same as those for the chemicals applied in broadcast applications—i.e., oral, dermal, and inhalation. For *B.t.k.*, however, the most directly relevant data used to characterize risk are based on actual applications of *B.t.k.* formulations where exposure is best characterized as an application rate. For the assessment of the potential for serious adverse effects, exposures are measured in colony forming units (cfu). LdNPV differs from all of the other agents in that no clear hazard potential can be identified. Thus, the most meaningful measure of exposure is in some respects moot. Those exposures that are quantified in the human health risk assessment for LdNPV are based on the mass of the formulation, Gypchek. Exposures to the gypsy moth itself are based on an indirect measure of exposure, egg masses/acre, because this is the expression of exposure that is used in the dose-response assessment.

Differences in the exposure assessments among the chemicals used in USDA programs primarily reflect differences in how the chemicals are applied, what routes of exposure are most substantial, and the nature of the available toxicity data. Diflubenzuron, tebufenozide, and disparlure may be applied in aerial broadcast applications that lead to multiple routes of exposure (oral, dermal, and inhalation). No chronic exposures for disparlure are conducted, however, because no chronic toxicity data are available on this chemical. DDVP, on the other hand, is used only in milk carton traps and exposures will be minimal under normal conditions, although much higher exposures are possible if the traps are not assembled properly or if individuals tamper with the traps.

3.2.2. Biological Agents

The exposure assessments for the biological agents —i.e., the gypsy moth, *B.t.k.*, and LdNPV differ substantially from each other, and these differences are largely dictated by the nature of the toxicity data available on each agent and the resulting dose-response assessments (Section 3.3.2).

3.2.2.1. Gypsy Moth – For the gypsy moth, the most direct and relevant measure of human exposure is probably the number of larvae per unit area or tree because it is contact with the larvae that causes skin irritation, the adverse effect typically associated with the gypsy moth. Nonetheless, the available dose-response data are based on studies in which exposure is

quantified as the number of eggs masses/acre. Accordingly, egg masses/acre is the exposure measure used in this risk assessment. As long as gypsy moth populations remain sparse, the larvae usually eat only a small proportion of the foliage of even their most favored host species, and contacts with people are rare. In such cases, egg masses generally do not exceed 50 egg masses/acre. During full-scale outbreaks, densities of about 5000 egg masses/acre are common and densities greater than 20,000 egg masses/acre are occasionally recorded. In such outbreaks, the numbers of gypsy moth larvae can reach up to 50,000 larvae per tree and exposure to the larvae will be essentially unavoidable for individuals near infested trees.

3.2.2.2. B.t.k. **–** The exposure assessment for *B.t.k.* is unusual in two respects. First, the most directly relevant data used to characterize risk are based on actual applications of *B.t.k.* formulations where exposure is best characterized as an application rate. As summarized in Section 3.3.2, epidemiology studies are available that report responses in populations after applications of *B.t.k.* in the range of those used in USDA programs to control the gypsy moth—i.e., 20-40 BIU/acre. Thus, these studies are used directly in the risk characterization and explicit exposure assessments and dose-response assessments are not needed.

Second, the apparent lack of a specific mechanism of toxicity for *B.t.k.* makes selecting the most appropriate measure of exposure somewhat arbitrary. The potency of *B.t.k.* is often expressed as BIU or FTU and exposures or application rates are expressed in units of BIU or FTU per acre. Although these units may be meaningful expressions of exposure for the gypsy moth, they are not necessarily or even likely to be a meaningful measures of human exposure. Exposure data are available, however, on *colony forming units* or cfu. When *B.t.k.* formulations are applied, either by aerial spray or ground spray, one or more viable spores contained in droplets or particulates is suspended in the air and deposited on sprayed surfaces. These droplets may be collected, either by air sampling or direct deposition, onto various types of filters. The filters are then cultured in a nutrient medium under conditions conducive to bacterial growth. As the bacteria grow, visible masses of bacteria, referred to as colonies, appear on the media. The significance of cfu as a measure of human exposure is limited because there is little indication that *B.t.k.* is a human pathogen. Consequently, the number of viable spores, albeit an important measure of exposure for the gypsy moth, does not appear to be toxicologicalally significant to humans. In this respect, cfu, like BIU, are of limited significance.

Nonetheless, at least for short-term exposures, cfu can be used as a practical measure of relative exposure to a *B.t.k.* formulation. Based on cfu, ground workers may be exposed to much higher concentrations of *B.t.k.* than other groups—i.e., 200,000-15,800,000 cfu/m^3. Much lower exposures, 400-11,000 cfu/m^3, have been measured in workers involved in aerial applications. During spray operations, members of the general public may be exposed to concentrations in the ranging from about 200-4000 cfu/m^3.

3.2.2.3. LdNPV **–** Given the failure to identify any hazard associated with LdNPV or the Gypchek formulation, there is little need to conduct a detailed exposure assessment for Gypchek. Gypchek contains gypsy moth parts, and these constituents, like the gypsy moth larvae

themselves, cause irritant effects in humans. The use of Gypchek, however, will not substantially increase the overall adverse effects of gypsy moth exposure in infested areas. On the contrary, the use of Gypchek will decrease the potential for human exposure to gypsy moth larvae by reducing larval populations. Based on simple physical processes associated with the application of any pesticide, it is possible to construct any number of exposure scenarios for Gypchek. The risk assessment for LdNPV focuses on one extreme exposure scenario involving the accidental spray of a home garden. While Gypchek is not intentionally applied to such vegetation, the inadvertent spray scenario is plausible. Based on this accidental exposure scenario, the estimated dose to an individual is 0.034 mg Gypchek/kg bw, with an upper range of 0.66 mg Gypchek/kg bw.

3.2.3. Chemical Agents

3.2.3.1. Diflubenzuron and Tebufenozide – Diflubenzuron and tebufenozide are applied in broadcast applications. The available data regarding the toxicity and environmental fate of these chemicals support a standard set of exposure scenarios involving worker exposure (both routine and accidental) and exposures of the general public to direct spray, dermal contact with contaminated vegetation, as well as the acute and longer-term consumption of contaminated food and water. For both of these chemicals, all exposure assessments are conducted at the maximum application rates. For diflubenzuron, all exposure assessments are conducted at the maximum single application rate for diflubenzuron of 0.0625 lb/acre, which is also the maximum amount that can be applied in a single season. The exposure assessments of tebufenozide are somewhat more elaborate because both single and multiple applications must be modeled—i.e., one or two applications at 0.12 lb/acre. While diflubenzuron is modeled at only the single maximum application rate, the exposure assessment for diflubenzuron is made elaborate by the quantitative consideration of the formation of 4-chloroaniline as an environmental metabolite. As noted in Section 3.1, the quantitative consideration of this metabolite is necessary because 4-chloroaniline is classified as a carcinogen and cancer risk is considered quantitatively in the risk characterization.

The exposure patterns for both diflubenzuron and tebufenozide are similar. For workers, three types of application methods are modeled: directed ground spray, broadcast ground spray, and aerial spray. In these general applications, the maximum exposures to workers are similar: 0.009 mg/kg/day for diflubenzuron and 0.02 mg/kg/day for tebufenozide. The differences in worker exposure levels merely reflect the differences in application rates for the two chemicals. Accidental dermal exposures for workers can be much higher: 0.4 mg/kg/day for diflubenzuron and 4 mg/kg/day for tebufenozide. These differences in exposure levels reflect the differences in the concentrations of the two chemicals used in field solutions as well as the differences in the estimated dermal absorption rates.

For members of the general public, the exposure profiles for diflubenzuron and tebufenozide are also similar. The maximum acute exposure levels for both chemicals are associated with contaminated water in an accidental spill scenario: doses of 1.5 mg/kg bw for diflubenzuron and 1.2 mg/kg bw for tebufenozide. Longer-term exposure to both agents, which involves the

consumption of contaminated fruit rather than water, will result in much lower levels of exposure: 0.002 mg/kg/day for diflubenzuron and 0.03 mg/kg/day for tebufenozide. Like workers, members of the general public can be at risk of dermal exposure to diflubenzuron or tebufenozide, and dermal exposure concentrations can be estimated quantitatively. Estimates of dermal exposure, however, are lower than estimates of oral exposure: a maximum of 0.05 mg/kg bw for diflubenzuron and about 0.4 mg/kg bw for tebufenozide.

Exposure assessments for 4-chloroaniline as an environmental metabolite of diflubenzuron are made only for members of the general public. Workers are not considered at risk because significant amounts of 4-chloroaniline are not likely to form during the application of diflubenzuron. For the general public, estimates of exposure to 4-chloroaniline from contaminated vegetation are likely to be about a factor of 50 below the corresponding estimates of exposure to diflubenzuron. The lower estimate of exposure to 4-chloroaniline is due to its expected rapid dissipation from diflubenzuron deposited on vegetation. In water, however, estimated concentrations of 4-chloroaniline are likely to be equal to or greater than anticipated water concentrations of diflubenzuron under certain circumstances. Finally, peak exposures to 4-chloroaniline differ from peak exposures to diflubenzuron in the environment, usually occurring at different times (later after the application of diflubenzuron) and under different conditions of precipitation. These differences are due to the relatively slow rate in the formation of 4-chloroaniline from diflubenzuron in soil.

3.2.3.2. Disparlure – Disparlure is like diflubenzuron and tebufenozide in that all three can be applied by aerial broadcast and multiple routes of acute and longer-term exposure are possible. The exposure assessment for disparlure, however, is much less elaborate than those for diflubenzuron and tebufenozide because of the very limited toxicity data base on disparlure. As discussed in Section 3.3 (Dose-Response Assessment), the U.S. EPA did not derive RfD values for acute or chronic exposure and the available toxicity data do not support the derivation of surrogate values. Thus, in the absence of toxicity data, an elaborate exposure assessment would not be useful in evaluating risk.

For disparlure, dermal exposure is most likely to be the predominant route for occupational exposure and is a possible route of exposure for the general public. A case report involving the accidental exposure of a worker to disparlure indicates that the only notable effect in the worker was the persistent attraction of gypsy moths. Since the available acute systemic toxicity of disparlure in mammals appears to be very low, the absence of dermal absorption data does not add significant uncertainty to this risk assessment. While dermal exposure of workers is expected to be non-toxic, dermal exposure is likely to cause the persistent attraction of gypsy moths.

Both workers and the public may be exposed to disparlure by inhalation, and the magnitude of the exposure can be estimated from available monitoring studies. At application rates more than 15 times the normal application rate (i.e., about 200 g a.i./acre compared with 29.1 g/acre), peak air concentrations ranged from 0.022 to 0.030 $\mu g/m^3$. Adjusted to the normal application rate,

these values correspond to about 0.003-0.004 $\mu g/m^3$, which is far below the air concentration of 5.0 mg/L —equivalent to 5000 µg/L or 5,000,000 $\mu g/m^3$—that did not cause mortality or signs of toxicity in experimental animals.

3.2.3.3. DDVP – Unlike the other chemicals used in gypsy moth control programs, DDVP is not applied in broadcast applications. DDVP is used only in a PVC strip that is placed in milk carton traps. Consequently, exposures of both workers and members of the general public should be negligible under normal conditions—i.e., the workers use proper procedures during assembly of the traps and members of the general public do not tamper with the traps. The risk assessment for DDVP does develop exposure scenarios for both workers and members of the general public to encompass improper handling of the DDVP strips by workers or tampering with the traps by members of the general public. These exposures, however, should be considered atypical, and some are extreme.

During assembly, the central estimate of dermal exposures in workers not wearing gloves leads to an absorbed dose of about 0.0008 mg/kg with a range of about 0.0003 mg/kg to 0.004 mg/kg. Inhalation exposures in workers may be highly variable depending on the ventilation rates in an enclosed space and the number of traps that are handled. Based on the handling and transport of 75 traps, inhalation exposures could be as high as 0.6 mg/m^3 in an enclosed and unventilated room and as high as 1.8 mg/m^3 in the passenger compartment of a vehicle. These exposure assessments are based on several site-specific and situation-specific assumptions intended to reflect plausible upper bounds of exposure.

Exposure assessments are also developed for children who might come in contact with an accidentally discarded or misplaced DDVP strip. Estimated dermal doses are much higher than those for workers: a central estimate of about 0.02 mg/kg with a range of 0.003-0.1 mg/kg. Oral exposures from a small child sucking on the pest strip are about a factor of 10 higher than dermal exposures: a central value of about 0.2 mg/kg with a range of 0.04-0.6 mg/kg.

Under normal circumstances, the use of DDVP in PVC strips is not likely to result in contamination of water or other materials that might be consumed by members of the general public. Nonetheless, an exposure assessment is developed for the accidental contamination of a small pond by a pest strip. In this scenario, dose estimates range from about 0.000003 to 0.00007 mg/kg with a central estimate of about 0.00001 mg/kg.

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview

A summary of the dose-response assessments for each of the agents covered in the risk assessment is given in Table 3-3. Dose-response assessments are typically based on an RfD, an estimate of a dose or exposure that is not likely to induce substantial adverse effects in humans. The RfD, in turn, is typically based on a NOAEL (no-observed-adverse-effect level) divided by an uncertainty factor. Risk is then characterized as a hazard quotient (HQ) which is the estimated level of exposure divided by the RfD. If the HQ is below unity—i.e., the exposure is less than the RfD —there is no credible risk. If the HQ is above unity, risk is characterized based on dose-response or dose-severity relationships. The quality of the dose-response assessment depends on the quality of the individual studies, the relevance of the studies to potential human exposures, and the strength of the dose-response relationship.

As in the exposure assessments (see Section 3.2), the dose-response assessments for the biological agents differ substantially from one another as well as from those of the chemical agents. The dose-response assessment for the gypsy moth itself is based on only one study; however, the study involves two human populations, and a demonstrates a clear dose-response relationship. Thus, confidence in the dose-response assessment is high. Two endpoints are considered for *B.t.k.*, irritant effects and more serious toxic effects. While the irritant effects are well documented, there is no apparent dose-response relationship and confidence in the dose-response is classified as medium. The dose-response assessment for more serious effects is based on a single study in mice that involves intranasal exposures. Although the study demonstrates a clear dose-response relationship, confidence in the dose-response assessment is low because intranasal exposures have marginal (if any) relevance to human exposure, the response was not independently replicated, and the observed response may be an artifact. For LdNPV, no endpoint of concern can be identified. Although the individual studies conducted on LdNPV are all somewhat dated, the weight of evidence for LdNPV and similar viruses clearly indicates the unlikelihood of systemic effects in humans after exposure to LdNPV. Thus, confidence in the dose-response assessment for LdNPV is classified as high.

Following standard practices in USDA risk assessments, risk assessment values available from U.S. EPA are adopted directly unless there is a compelling basis for doing otherwise. This approach is taken because the U.S. EPA will typically devote substantial resources and expertise to the development of risk assessment values and it is not feasible to duplicate this effort in risk assessments prepared for the USDA. In addition, the U.S. EPA has the legislative mandate to develop risk values for pesticides and it is sensible for the USDA to administratively defer to U.S. EPA in this area. When risk values are not available from U.S. EPA, the methods used by U.S. EPA are employed to derive surrogate values. Except for disparlure, chronic RfD values are available from U.S. EPA and these values are used directly. For 4-chloroaniline, the U.S. EPA derived a cancer potency factor as well as a chronic RfD, and these values are used directly in the risk assessment. For DDVP, the U.S. EPA derived an acute RfD, and this value is also adopted in the current risk assessment. A complication with DDVP, however, is that this agent is contained within a PVC strip, which substantially impacts its bioavailability. In order to consider

this matter quantitatively, a single and somewhat marginal study regarding the toxicity of DDVP in a PVC strip is used, and confidence in the dose-response assessment is, in turn, marginal. Unlike all of the other chemicals considered in this comparative risk assessment, very little toxicity data are available on disparlure. The U.S. EPA did not derive an RfD for this chemical, and the available toxicity data are insufficient to derive a surrogate RfD. Thus, confidence in the dose-response assessment for disparlure is marginal.

3.3.2. Biological Agents

3.3.2.1. Gypsy Moth – The dose-response assessment for human health effects is based on reports of skin irritation in two populations: one with low exposure (an average of 32 egg masses/acre) and the other with high exposure (an average of 3809 egg masses/acre). The low-exposure group exhibited no increase in skin irritation. Accordingly, 32 egg masses/acre is taken as a NOAEL (no-observed-adverse-effect level) for humans and is used as a surrogate RfD for exposure to the gypsy moth in a manner analogous to the use of RfD values for control agents. The high exposure group had a significant increase in skin irritation, and, based on a dose-response model developed by U.S. EPA, egg mass densities up to128 egg masses/acre are not likely to cause a detectable increase in skin irritation or rashes.

While the dose-response relationship is based on only two exposure levels, the strength of the dose-response relationship is strong. Typically, an association is judged to be statistically significant if the *p*-value (the probability that the association occurred by chance) is 0.05 or less. For the study on which these dose-response relationships are based, p-values are on the order of 0.0004 or less for most groups. The only exception involves individuals over the age of 59 years. In this group, it is unclear if the lack of a significant response is related to a lesser sensitivity to the gypsy moth or less exposure—i.e., less time spent outdoors.

In addition to these quantitative estimates of response, the severity of the response is important, particularly in a comparison of effects caused by exposure to the gypsy moth and effects caused by exposure to the agents used to control the gypsy moth. Dermal responses to the gypsy moth are sufficiently severe to have generated numerous case reports. While precise statistics are not available, it does appear that the severity of the skin irritation is sufficient to cause appreciable numbers of affected individuals to seek medical care. While exposure to the gypsy moth is associated with irritation to the eyes and respiratory tract, quantitative dose-response relationships for these endpoints cannot be developed.

3.3.2.2. B.t.k. – Two types of dose-response assessments are presented for *B.t.k.*, one for irritant effects and the other systemic toxicity. There is relatively high confidence that formulations of *B.t.k.* will cause various types of irritant effects in humans and experimental animals; however, confidence in the quantitative assessment of these effects is limited by a very weak dose-dependency in the incidence of the response. The quantitative assessment for systemic toxic effects is extremely tenuous because it is based on a very conservative interpretation of a single study using a route of exposure (intratracheal instillation) that typically is not used in quantitative risk assessments.

The estimate for irritant effects is actually a set of observations rather than a formal dose-response assessment. Several epidemiology studies were conducted after *B.t.k.* applications at rates within the range of those used in USDA programs to control the gypsy moth—i.e., 20-40 BIU/acre. Two key epidemiology studies, one involving workers (Cook 1994) and the other involving members of the general public (Petrie et al. 2003), suggest that irritant effects, particularly throat irritation, may be reported in groups of humans during or after applications of *B.t.k.* In the worker study, the data demonstrate a statistically significant increase in the incidence of irritant effects in workers. The significantly increased effects include generalized dermal irritation (dry or itchy skin and chapped lips), irritation to the throat, and respiratory irritation (cough or tightness). Furthermore, the overall incidence of all symptoms combined was increased significantly among the workers, compared with the controls . In the study involving the general public, several types of irritant effects are reported; however, the only effect that is clearly statistically significant involves throat irritation ($p=0.002$).

Confidence in accepting whether these reports are biologically significant, however, is reduced by the apparent lack of a strong dose-response relationship. The workers were exposed to up to about 16 million cfu/m^3 and the reported incidence of throat irritation is about 24%. In the study involving members of the general public, no measures of exposure are given. Based on monitoring data from similar applications, however, it is likely that members of the general public may have been exposed to air concentrations ranging from approximately 100 to 4000 cfu/m^3 during or shortly after aerial applications of *B.t.k.* This range is a factor of 3950 to 158,000 less than exposures in the worker study. The apparent incidence of throat irritation in the study on members of the general public, however, is about 19%. Thus, while these much lower exposures lead to a somewhat lesser response, the dose-response relationship appears weak. Nonetheless, these studies are taken together to characterize risk semi-quantitatively, as discussed further in Section 3.4.

There is essentially no information indicating that oral, dermal, or inhalation exposure to *B.t.k.* or *B.t.k.* formulations will cause serious adverse health effects. Extremely severe inhalation exposures that coat the test species with commercial formulations of *B.t.k.* are associated with decreased activity, discolored lungs, and other effects but not mortality. Although the animal data are consistent with data regarding human exposure *B.t.k.*, the animal studies are all based on single concentrations and cannot be used in a meaningful dose-response assessment.

Few studies (David 1990; Hernandez et al. 1999,2000) report mortality after exposure to *B.t.k.,* and these studies, while related to inhalation toxicity, involve atypical routes of exposure. One such study (David 1990) was conducted on a *B.t.k.* Dipel formulation after intratracheal instillations. Intratracheal instillations of bacteria are analogous to inhalation exposures in that the bacteria is essentially inserted into the lungs. Toxic responses including death were observed in treated animals, and the time-to-clearance (estimated from linear regression) was prolonged. Hernandez et al. (1999, 2000) assayed the toxicity of *B.t.k.* after intranasal instillations in mice. This method of dosing is also analogous to inhalation exposures in that the material is deposited in nasal passages and the *B.t.k.* is gradually transported to the lungs by inhalation. Doses of 10^2,

10^4, and 10^6 cfu/mouse caused only local inflamation. A dose of 10^8 cfu/mouse resulted in 80% lethality.

In terms of the human health risk assessment, the data from Hernandez et al. (1999, 2000) are not directly useful. Furthermore, the route of exposure (intranasal instillation) makes any use of these data somewhat tenuous. Concern with the use of this atypical route of exposure in a dose-response assessment is exacerbated because the Hernandez et al. (2000) study does not specify whether or not the instillations were adjusted to a constant volume. If the installations were not adjusted to a constant volume, it is possible that the observed dose-response relationship could be due to differences in volumetric bronchial obstruction or a combination of bronchial obstruction and *B.t.k.*

Notwithstanding these reservations, the Hernandez et al. (1999, 2000) studies provide the best dose-response data available in experimental mammals. Based on a consideration of the Hernandez et al. (2000) study and the estimates of equivalent human exposures, it seems plausible that cumulative exposures up to $1.4x10^{10}$ cfu/m^3 x hour will not cause adverse effects in humans. This estimate is supported by the worker study (Cook 1994) from which an apparent NOAEL of $3x10^8$ cfu/m^3 x hours for adverse health effects in humans can be calculated, and this value is used quantitatively to characterize the potential for serious adverse effects in humans.

3.3.2.3. LdNPV **–** The dose-response assessment for LdNPV and its formulation as Gypchek is extremely simple, compared with the other biological and chemical agents, except disparlure, used to control the gypsy moth. Due to the lack of systemic toxic effects associated with any plausible route of exposure (i.e., oral, dermal, or inhalation), the U.S. EPA did not derive an acute or chronic RfD for Gypchek. Although this approach is reasonable, the risk assessment for LdNPV, which is used in the EIS, derives a surrogate acute RfD of 26 mg/kg bw. The surrogate RfD, which is based on an experimental acute NOAEL of 2600 mg/kg bw in rats and an uncertainty factor of 100, provides a quantitative basis for comparison between the extremely low risks associated with the application of Gypchek and the risks posed by the other gypsy moth control agents. Confidence in this value is limited because no adverse effect levels were identified—i.e., the true NOAEL for Gypchek may be higher than 2600 mg/kg. This uncertainty in the LdNPV risk assessment is relatively minor, given that even extreme exposures are far below any level of concern (Section 3.4).

Technical grade Gypchek is an eye irritant. While not quantitatively considered in the risk assessment, the distinction between the irritant properties of technical grade Gypchek and the lack of eye irritation associated with Gypchek formulations applied in the field is emphasized in order to highlight areas in which prudent handling practices are likely to be most important.

3.3.3. Chemical Agents

3.3.3.1. Diflubenzuron and Tebufenozide **–** As discussed in the hazard identification and the exposure assessment, diflubenzuron and tebufenozide are similar to one another in terms of their toxicological profiles. Both chemicals were tested in a similar and relatively standard set of

toxicity studies required by the U.S. EPA for the registration of pesticides. Their most sensitive endpoint, hematological effects (including methemoglobin formation and several other endpoints characteristic of hemolytic anemia) was observed in all mammalian species tested.

Quantitatively, the similarities between diflubenzuron and tebufenozide are further expanded and even more striking in the dose-response assessment. The U.S. EPA derived RfDs for both compounds and the values are virtually identical: 0.02 mg/kg/day for diflubenzuron and 0.018 mg/kg/day for tebufenozide. Even this minor difference is an artifact of rounding. The U.S. EPA agency-wide workgroup, which derived the RfD for diflubenzuron, typically rounds all RfDs to one significant place. The U.S. EPA Office of Pesticides, which derived the RfD for tebufenozide, often reports RfDs to two significant places. If the agency-wide criteria had been applied to tebufenozide, the two RfDs would be identical—i.e., 0.02 mg/kg/day. Since the molecular weights of diflubenzuron (310 g/mole) and tebufenozide (352 g/mole) are so similar, the RfDs would be identical even when expressed in moles—i.e., $7x10^{-5}$ mMoles/kg/day for diflubenzuron and $5x10^{-5}$ mMoles/kg/day for tebufenozide.

The RfDs for both chemicals are based on dietary studies in rats, and the respective NOAELs are quite similar: 2 mg/kg/day for diflubenzuron and 1.5-2.4 mg/kg/day for tebufenozide. Again, these minor differences are an artifact of the way in which the dietary concentrations (i.e., mg agent/kg diet) used in the studies were converted to dose estimates expressed as mg/kg bw/day based on food consumption. Both RfDs are also based on an uncertainty factor of 100, a factor of 10 for interspecies differences—i.e., extrapolation of animal data to humans—and a factor of 10 for intraspecies variability—i.e., individuals who might be most sensitive to the chemical. For both chemicals, the U.S. EPA determined that an additional uncertainty factor of 10 for the protection of infants and children, a factor that must be considered under the Food Quality Protection Act (FQPA), is not required. Finally, confidence in both RfDs is high, which is stated explicitly in the Agency wide RfD for diflubenzuron and is implicit in the discussion of the chronic RfD for tebufenozide derived by the U.S. EPA Office of Pesticides—i.e., no data gaps are identified.

The acute dose-response assessments on diflubenzuron and tebufenozide prepared by U.S. EPA are similar in that the U.S. EPA elected not to derive an acute RfD for either compound. This approach is taken because the agency concluded that no endpoint for acute dietary exposure could be identified for either chemical. U.S. EPA identifies an acute NOAEL of 10,000 mg/kg bw for diflubenzuron and an acute oral NOAEL of 2000 mg/kg bw for tebufenozide. For the USDA risk assessments on gypsy moth control agents, surrogate acute RfDs are derived for both chemicals according to the methods typically employed by the U.S. EPA, because many areas of greatest concern involve potential acute effects after accidental or incidental exposures.

For diflubenzuron, a surrogate acute RfD of 100 mg/kg could be derived using the NOAEL of 10,000 mg/kg identified by U.S. EPA. A more conservative approach is taken, however, using the NOAEL of 1118 mg/kg from an acute study (single dose) in which Dimilin 4L, a formulation containing petroleum oil, was used. The resulting surrogate acute RfD is 11 mg/kg. A similar

approach is taken for tebufenozide. Rather than using an acute NOAEL of 2000 mg/kg, a NOAEL of 1000 mg/kg/day in pregnant rats and rabbits, identified by U.S. EPA, is used to derive a surrogate acute RfD of 10 mg/kg/day. Like the chronic RfDs, the acute RfDs are nearly identical.

The dose-response assessment for diflubenzuron is somewhat more complicated than that for tebufenozide because of the need to consider 4-chloroaniline quantitatively. As noted in the hazard identification (see Section 3.1), 4-chloroaniline is an environmental metabolite of diflubenzuron and 4-chloroaniline has been classified as a potential human carcinogen. The U.S. EPA derived a chronic RfD for 4-chloroaniline of 0.004 mg/kg/day, and this value is used to characterize risks from 4-chloroaniline for longer-term exposures. This RfD is based on a chronic oral LOAEL of 12.5 mg/kg/day using an uncertainty factor of 3000, three factors of 10 for interspecies extrapolation, sensitive subgroups, and the use of a LOAEL with an additional factor of 3 due to the lack of reproductive toxicity data. As with diflubenzuron, the U.S. EPA has not derived an acute RfD for 4-chloroaniline. For 4-chloroaniline, a conservative approach is taken in which a surrogate acute RfD of 0.03 mg/kg is based on a subchronic (90-day) NOAEL of 8 mg/kg/day. Consistent with the approach taken by U.S. EPA for the chronic RfD, an uncertainty factor of 300 is used. For cancer risk, the U.S. EPA proposes a human cancer potency factor for 4-chloroaniline of 0.0638 $(mg/kg/day)^{-1}$. This potency factor is used to calculate a dose of $1.6x10^{-5}$ mg/kg/day that could be associated with a plausible upper limit of cancer risk of 1 in 1 million.

3.3.3.2. Disparlure – As noted in the hazard identification (see Section 3.1.3.3), the U.S. EPA does not require extensive testing of insect pheromones, including disparlure. This approach is taken because insect pheromones are generally regarded as nontoxic to mammals and because these pheromones are commonly employed in very low environmental concentrations. While the merits of this approach may be argued, the result is that there is little information regarding the toxicity of disparlure, and no RfD values, acute or chronic, have been or can be derived.

The only information that can be used to assess the consequences of exposure to disparlure are LD_{50} or LC_{50} values: an oral LD_{50} value greater than 34,600 mg/kg; a dermal LD_{50} value greater than 2025 mg/kg, and an inhalation LC_{50} value greater than 5 mg/L · 1 hour. Notably, each of the values is expressed as "greater than". In other words, less than half of the organisms died at the specified exposure. In the case of disparlure, these values are actually NOEC values for mortality in that none of the animals died during any of the exposures.

3.3.3.3. DDVP – Like diflubenzuron and tebufenozide, and perhaps to an even greater extent, DDVP has an extensive toxicology data base that has been evaluated by numerous government organizations, including U.S. EPA, the Agency for Toxic Substances and Disease Registry (ATSDR), National Institute for Occupational Safety and Health (NIOSH), Occupational Safety and Health Administration, and the World Health Organization. As noted above, these sources are used when possible for selecting levels of acceptable exposure. Because all of the scenarios

considered in this risk assessment involve only acute exposures, only acute exposure criteria are considered.

The acute RfD established by the U.S. EPA for oral and dermal exposure to DDVP, 0.0017 mg/kg, is used for the risk characterization. The RfD is based an acute oral NOAEL of 0.5 mg/kg from a rat study, and the application of an uncertainty factor of 300. Acute exposure criteria proposed by other groups are comparable to but somewhat higher than the acute RfD. Because some of the accidental acute exposures may substantially exceed the acute RfD, some attempt is made to characterize the consequences of high oral exposures. A human NOAEL of 1 mg/kg for AChE inhibition has been identified. While this NOAEL is not used to modify the acute RfD, it can be used to assess plausible consequences of exceeding the RfD. The human data on DDVP, although extensive, are not sufficient to identify a minimal lethal dose. For the current risk assessment, the lowest reported lethal dose (16 mg/kg) is used to assess the plausibility of observing serious adverse effects in cases of accidental overexposure to DDVP.

A number of inhalation criteria are available for DDVP. Since potentially significant inhalation exposures are likely only in workers, the occupational exposure criterion of 0.1 mg/m^3 proposed by American Conference of Governmental Industrial Hygienists is used. This value is a factor of 10 below the occupational criteria proposed by NIOSH and OSHA.

A major factor and a major complication in the dose-response assessment of DDVP involves the formulation of DDVP in a PVC strip. Some of the accidental exposures considered in this risk assessment involve a small child gaining access to a DDVP-PVC strip and being subject to both oral and dermal exposure. While there is little doubt that the PVC strip will slow the rate of exposure and reduce the risk, this is extremely difficult to quantify. Despite the availability of numerous studies regarding the toxicity of DDVP itself, the number of studies regarding the toxicity of DDVP-PVC strips is relatively small. By far the most relevant study is that conducted by Stanton et al. (1979), which clearly indicates that DDVP in a PVC formulation will be much less toxic than unformulated DDVP. The extent of the difference in toxicity can only be semi-quantitatively characterized. For unformulated DDVP, the LD_{50} value was 157 (113–227) mg/kg with no mortality observed at 56 mg/kg. For the DDVP-PVC formulation, no deaths occurred at doses of up to 1000 mg/kg, although signs of toxicity consistent with AChE inhibition were observed at doses of 320 and 1000 mg/kg. Neither tremors nor salivation were observed at doses of 240 or 180 mg/kg. Stanton et al. (1979) do not provide comparative data on the extent of AChE inhibition in unformulated DDVP and the DDVP-PVC formulation.

3.4. RISK CHARACTERIZATION

3.4.1. Overview

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. For systemic toxic effects, risk characterizations are presented as hazard quotients (HQs). A hazard quotient is the ratio of a projected level of exposure divided by some index of an acceptable exposure, such as an RfD. If the HQ is substantially less than one – i.e., the level of exposure is less than the level of acceptable exposure—there is no apparent cause for concern. If the hazard quotient is greater than unity, there is cause for concern.

Because the hazard quotient does not describe dose-response or dose-severity relationships, a comparison of the magnitudes of the hazard quotients among different agents may not be a reliable index of relative risk and other types of information need to be considered. Hazard quotients that are close to a level of concern—i.e., between about 0.1 and 10—may be more difficult to interpret because of uncertainties in both the exposure estimates as well as the dose-response relationships. While the range from 0.1 to 10 is somewhat arbitrary in terms of classifying the nature of concern, this is similar to the approach recently adopted by ATSDR (2004) in which concern for interactions of chemicals is triggered when individual hazard quotients exceed a value of 0.1.

In order to reflect these gradations of concern in the general interpretation of hazard quotients, the comparative risk characterization is not organized by biological and chemical agents (as in the previous sections) but is organized by the nature of the hazard quotients: agents of marked concern (HQ>10), agents of marginal concern (HQ>0.1 but <10), and agents of ***minimal*** concern (HQ<0.1). The word ***minimal*** is emphasized because of the inherent limitation in all risk assessments. Risk assessments can never prove absolute safety—i.e., it is impossible to prove the negative, that something does not exist, in this case risk. Risk assessments, however, can be and are used to determine whether or not there is a basis for asserting that risk is plausible.

An overview of the comparative risk characterization is summarized in Table 3-4 and illustrated in Figure 3-1. Of the agents considered in this risk assessment, the gypsy moth and DDVP are clearly agents of marked concern, although the nature of the concerns is different. If the gypsy moth is not controlled, population outbreaks will occur and humans will be exposed to large numbers of gypsy moth larvae. If this occurs, a substantial number of individuals will experience irritant effects that are sufficiently severe to cause these individuals to seek medical attention. No more serious effects are likely. For DDVP, the potential for risk is clear but the likelihood of observing risk seems to be remote. Under normal conditions and proper handling, exposures to DDVP will be negligible and risk will be inconsequential. Workers who mishandle a DDVP-PVC strip or members of the general public who handle a DDVP-PVC may be exposed to levels of DDVP that are far above levels that would be considered acceptable. While such exposures clearly should be avoided, it seems unlikely that they would result in frank signs of toxicity. This conclusion is consistent with human experience in the use of DDVP resin strips.

Diflubenzuron and tebufenozide are agents of marginal concern. Under most foreseeable conditions of exposure—i.e., exposure scenarios that might be characterized as typical—levels of exposure will be far below levels of concern. At the upper ranges of plausible exposure— levels that might be characterized as extreme—the hazard quotients for diflubenzuron approach a level of concern (HQs between 0.1 and 0.5 for both diflubenzuron and its 4-chloroaniline metabolite). For tebufenozide, the highest hazard quotient is 1.5, indicating that, although unlikely to cause overt signs of toxicity, the exposure would be characterized as undesirable. The somewhat higher hazard quotients for tebufenozide are attributed solely to the higher application rates for this compound, compared with diflubenzuron.

Among the agents of minimal concern, *B.t.k.* is somewhat problematic. Based on the risk for serious adverse effects, there is clearly no cause for concern (the highest HQ is 0.04). As discussed in the dose-response assessment, this lack of concern is reenforced by a very aggressive and protective interpretation of the available toxicity data. Nonetheless, there is some residual concern with irritant effects. These effects are quite plausible in accidental cases of gross overexposure—e.g., splashing a formulation into the eye. These kinds of concern are minimal and are common to almost all chemical or biological agents. The more troubling concern involves studies of workers and non-workers who report irritant effects, primarily throat irritation. Whether or not these effects should be attributed to the *B.t.k.* exposure is unclear.

The risk characterization for LdNPV and disparlure is unequivocal. Based on the available information, there is no plausible basis for concern that exposure will cause serious adverse effects. Again, various accidental exposures, such as splashing the agent into the eyes, might cause transient irritant effects.

3.4.2. Agents of Marked Concern

3.4.2.1. Gypsy Moth – Although the quantitative dose-response assessment is based on only one study, the study demonstrates a clear dose-response relationship and is supported by less quantitative reports of irritant effects associated with exposure to the gypsy moth as well as other lepidopteran larvae. In sparse to moderate infestations—i.e., egg mass densities of more than 500 egg masses/acre—adverse effects involving skin irritation are not likely to be detectable in populations of exposed humans. Nonetheless, some individuals who have contact with gypsy moth larvae might develop skin irritation. In heavy gypsy moth infestations—i.e., from more than 500 to 5000 egg masses/acre—the occurrence of adverse skin reactions is expected to be hight, and the effects are likely be severe enough to cause some individuals to seek medical attention. In extreme outbreaks—i.e., greater than 5000 egg masses/acre— the effects will be qualitatively similar to those of severe infestations but may affect up to one-third of the population. Heavy infestations or extreme outbreaks may cause ocular and respiratory effects in some people; nonetheless, there is no way to quantify the likelihood of observing these effects. Similarly, severe infestations are often considered to be a nuisance and cause aesthetic damage to the environment. Both of these factors can lead to stress in some individuals. Young children may be a group at special risk from effects of gypsy moth exposure; however, it is not clear whether children are more sensitive than adults to the effects of gypsy moth exposure or whether

responses in children appear greater because children spend more time outdoors compared with adults.

3.4.2.2. DDVP – In most cases, exposures to both workers and members of the general public should be negligible. If workers take prudent steps to limit both dermal and inhalation exposures, the likelihood of exposures to DDVP reaching a level of concern appears to be very low. Similarly, members of the general public should not be exposed to substantial amounts of DDVP. The DDVP is contained within a PVC strip to ensure that the active ingredient is slowly released over a long period of time. The strip, in turn, is placed within a trap and the trap is placed so that it will not be accessed except in the case of intentional tampering or trap monitoring.

Nonetheless, the risk assessment for DDVP develops exposure scenarios for both workers and members of the general pubic, which are intended to illustrate the potential effects of mishandling or tampering with DDVP strips. For workers, the greatest risks are associated with inhalation exposures from assembling the traps in enclosed and poorly ventilated spaces or transporting the traps in the passenger compartments of vehicles. These risks can be readily avoided. Dermal exposures can also lead to lesser but sill undesirable levels of exposure. For members of the general public, all of the exposure scenarios are accidental and some are extreme. The most likely of these is the accidental contamination of a small body of water. This scenario leads to exposures that are below the level of concern by a factor of about 25. If a child were to come into contact with a DDVP strip, however, both dermal and oral exposures could substantially exceed a level of concern. Although such exposures clearly should be avoided, it seems unlikely that they would result in frank signs of toxicity. This conclusion is consistent with human experience in the use of DDVP resin strips.

3.4.3. Agents of Marginal Concern

3.4.3.1. Diflubenzuron – The risk characterization for potential human health effects associated with the use of diflubenzuron is relatively unambiguous: none of the hazard quotients reach a level of concern at the highest application rate that could be used in USDA programs. In that many of the exposure assessments involve very conservative assumptions—that is, assumptions that tend to overestimate exposure—and because the dose-response assessment is based on similarly protective assumptions, there is no plausible basis for concluding that this use of diflubenzuron poses a hazard to human health.

Notwithstanding the above assertion, it is worth noting that the greatest relative risk concerns the contamination of water with 4-chloroaniline rather than exposure to diflubenzuron itself. The highest hazard quotient for diflubenzuron is 0.1, a factor of 10 below a level of concern. Since this hazard quotient is based on toxicity, an endpoint that is considered to have a population threshold, it is reasonable to assert that the risk associated with exposure to diflubenzuron is essentially zero.

Such is not the case with 4-chloroaniline, which is classified as a probable human carcinogen and is an environmental metabolite of diflubenzuron. For 4-chloroaniline, the highest hazard quotient is 0.4, below the level of concern by a factor of only 2.5. The scenario of greatest concern involves cancer risk from drinking contaminated water. This risk would be most plausible in areas with sandy soil and annual rainfall rates ranging from about 50 to 250 inches. The central estimate of the hazard quotient for the consumption of water contaminated with 4-chloroaniline and based on a cancer risk of 1 in 1million is 0.09, which is 10 times lower than the level of concern.

3.4.3.2. Tebufenozide – The similarities between tebufenozide and diflubenzuron have been emphasized throughout this comparative risk assessment. As noted in the dose-response assessment, the toxicities of these two compounds are virtually identical. While both diflubenzuron and tebufenozide are classified as agents of marginal concern—i.e., risk quotients between 0.1 and 10 —tebufenozide does exceed the level of concern, whereas diflubenzuron does not. This difference is due to the higher application rates that may be used with tebufenozide. These higher application rates for tebufenozide increase the levels of exposure, which results in somewhat higher hazard quotients for tebufenozide, compared with diflubenzuron.

Nonetheless, as with diflubenzuron, there is no clear indication that adverse effects are likely to result from exposure to tebufenozide. At the maximum application rate considered in this risk assessment, two applications at 0.12 lb/acre spaced three days apart, there is little indication that adverse effects on human health are likely and only one scenario exceeds a risk quotient of 1. Based on central estimates of exposure— those that might be considered typical and expected—hazard quotients including workers and members of the general public range from 0.00003 to 0.03, below a level of concern by factors ranging from approximately 30 to 33,000. At the upper range of plausible exposures, the hazard quotient for ground spray workers reaches a level of concern—i.e., a hazard quotient of 1. For members of the general public, the upper range of exposure leads to a hazard quotient of 1.5 for the longer-term consumption of contaminated vegetation following two applications at 0.12 lb/acre. Because of the linear relationship between exposure and application rate, two applications at 0.08 lb/acre would reach but not exceed a level of concern. With a single application at the maximum rate of 0.12 lb/acre, the hazard index is 0.8, below the level of concern. While the longer-term consumption of contaminated vegetation is probably not a likely scenario, it is a standard exposure scenario used in USDA risk assessments to consider the longer-term consumption of food items, like berries, that might be sprayed during the broadcast application of a pesticide. This risk assessment suggests that two applications at 0.08 lb/acre or more should be avoided in areas where members of the general public might consume contaminated fruits or other contaminated vegetation.

3.4.4. Agents of Minimal Concern

3.4.3.1. B.t.k. – The risk characterization regarding exposure to *B.t.k.* and its formulations is generally consistent with that of the previous USDA risk assessment as well as more recent risk assessments conducted by the U.S. EPA and the World Health Organization: *B.t.k.* and its

formulations are likely to cause irritation to the skin, eyes, and respiratory tract; however, serious adverse health effects are implausible. Whether irritation is caused by *B.t.k.* in typical field applications used to control the gypsy moth is uncertain. While epidemiology studies involving self-reporting of symptoms do suggest that reports of irritant effects are to be expected, the biological plausibility of these effects is called into question because of an insubstantial dose-dependency for the irritant effect.

B.t.k. applications to control or eradicate the gypsy moth are not expected to cause serious adverse health effects in humans. At the extreme upper range of exposure in ground workers, exposure levels are estimated to be below the functional human NOAEL for serious effects by a factor of 25. For members of the general public, exposure levels are estimated to be below the functional human NOAEL by factors ranging from about 28,000 to 4,000,000 [4 million]. This assessment is based on reasonably good monitoring data, conservative exposure assumptions, and an aggressive and protective use of the available toxicity data. Based on these data, it is not likely that overt signs of toxicity will be observed in any group— ground workers, aerial workers, or members of the general public—exposed to *B.t.k.* as the result of gypsy moth control and eradication programs conducted by the USDA.

There is no documented evidence of a subgroup of individuals who are more sensitive than most members of the general public to *B.t.k.* formulations. According to a recent epidemiology study, asthmatics are not likely to be affected adversely by aerial applications of *B.t.k.* The literature on *B.t.k.* includes one anecdotal claim of a severe allergy to a carbohydrate in a *B.t.k.* formulation; however, neither the claim nor observations of similar effects are substantiated in the available published epidemiology studies. On the other hand, *B.t.k.* formulations are complex mixtures, and the possibility that individuals may be allergic to some of the components in the formulations is acknowledged by a state health service.

As noted in Section 3.1, pre-treatment with an influenza virus substantially increased mortality in mice exposed to various doses of *B.t.k.* Although the relevance of this observation to public health cannot be assessed well at this time, the viral enhancement of *B.t.k.* toxicity is likely to be an area of further study in the coming years.

3.4.3.2. LdNPV (Gypchek) **–** There is no plausible basis for concern that either workers or members of the general public are at risk of adverse effects from the use of Gypchek to control the gypsy moth. This statement follows from the failure to identify any hazard associated with exposures to Gypchek or LdNPV and is essentially identical to the risk characterization given by the U.S. EPA.

As discussed in both the exposure and dose-response assessments, the current risk assessment extends the U.S. EPA risk assessment by proposing a surrogate acute RfD and presenting a very conservative exposure assessment based on the accidental spray of a home garden. This approach is taken simply to facilitate the comparison of risks (or lack of risk) associated with Gypchek to the risks associated with other agents used to control the gypsy moth. Based on a

relatively standard dose-response assessment and very conservative exposure assumptions, plausible exposures to Gypchek are below a level of concern by factors ranging from about 50 to more than 750. While more typical exposures—i.e., incidental exposure to Gypchek in water or air—are not provided, they will be substantially less than the range of accidental exposure scenarios used to quantify risk.

3.4.3.3. Disparlure – Although there are studies regarding the acute toxicity of disparlure in laboratory animals, the lack of subchronic and chronic toxicity data precludes a quantitative characterization of risk. The available data regarding the acute toxicity of disparlure indicate that the potential hazard from exposure to the compound is low.

The reliance on acute toxicity data introduces uncertainties into the risk assessment of disparlure that are quite different from the other better studied agents, and these uncertainties cannot be quantified. Other uncertainties in this analysis are associated with the exposure assessment and involve environmental transport and dermal absorption. These uncertainties are relatively minor compared with the lack of subchronic or chronic toxicity data. Thus, while there is no reason to believe that longer-term exposure to disparlure will produce adverse effects, this assumption can not be substantiated due to the lack of chronic toxicity data. The significance of this uncertainty is at least partially offset by the very low exposures that are plausible given the limited use of disparlure. For example, as noted in the dose-response assessment, inhalation exposures of mice to 5 mg/L (5,000,000 $\mu g/m^3$) for 1 hour caused no mortality or signs of toxicity. As noted in the exposure assessment, likely concentrations of disparlure in air after applications comparable to those used in programs to control the gypsy moth are likely to be on the order of 0.004 $\mu g/m^3$, a factor of 1,250,000,000 (1.25 billion) below the apparent NOEC for acute toxicity. This relationship is consistent with the general assumption made by the U.S. EPA that exposures to insect pheromones will be far below levels of concern (U.S. EPA 2004).

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview

An overview of the comparative hazard identification is given in Table 4-1. Unlike the human health risk assessment, in which the potential effects of the biological agents are similar, each of the ecological effects profiles of the biological agents considered in this risk assessment is quite distinct. The principal effect of the gypsy moth is damage to sensitive trees, which can be substantial. Because of the obvious importance of vegetation to the existence and habitat of most animals, defoliation by the gypsy moth will have numerous secondary effects in many other groups of organisms. There is, however, no indication that the gypsy moth will have direct effects on groups of organisms other than sensitive plants. LdNPV, on the other hand, is unlikely to have effects on species other than the gypsy moth. *B.t.k.* is toxic to nontarget *Lepidoptera* as well as the gypsy moth and some other lepidopteran species, but is unlikely to have direct effects on other groups of organisms. Thus, the potential effects of all of the biological agents are considered relatively specific, with LdNPV showing the greatest degree of specificity (only the gypsy moth), followed by the gypsy moth itself (several types of plants) and *B.t.k.* (several types of *Lepidoptera*).

The chemical agents also differ in specificity: disparlure is most specific, tebufenozide is relatively specific to *Lepidoptera*, diflubenzuron is less specific and may affect many arthropods, and DDVP is a nonspecific biocide toxic to most groups of animals. As a pheromone, disparlure is almost as specific as LdNPV. It will attract the gypsy moth and two other closely related species, the nun moth (*Lymantria monacha*) and the pink gypsy moth (*Lymantria fumida*). Like the gypsy moth, both of these *Lymantria* species are forest pests, and adverse effects on these species are not a substantial concern for this risk assessment. In addition, the pink gypsy moth is native to Japan and is not found in the United States. A major qualification regarding the specificity of disparlure is the limited amount of information available on nontarget species. The data that are available indicate that the relative toxicity of disparlure to *Daphnia magna*, a commonly used test species in aquatic toxicity studies, is high. Diflubenzuron and tebufenozide are clearly toxic to mammals and at least some arthropods. In mammals, exposure to either chemicals causes adverse effects in blood (methemoglobinemia), as discussed in the human health risk assessment. In terrestrial and aquatic arthropods, exposure to either chemical interferes with growth and development. Because of differences in the mechanism of action of diflubenzuron and tebufenozide, the toxicity of tebufenozide appears to be somewhat more selective. For instance, effects in birds have been clearly demonstrated for tebufenozide but not for diflubenzuron. Nonetheless, it is plausible to speculate that both diflubenzuron and tebufenozide are likely to cause adverse hematological effects in birds, similar to those observed in mammals exposed to these chemicals. In terms of its mechanism of action, DDVP is a general neurotoxin. In all animals that have nervous systems that involve acetylcholinesterase (AChE) and use acetylcholine (ACh) as a neurotransmitter (a substance necessary to make the nerves work properly), DDVP will be toxic and sufficiently high exposures to DDVP will be lethal. The definition of *sufficiently high*, however, is critical and variable. Although DDVP is not selective

mechanistically, differences in sensitivity among species are substantial. Insects are much more sensitive than mammals or other higher organisms to DDVP. This difference in sensitivity is what characterizes DDVP as an effective insecticide that can be used safely.

4.1.2. Biological Agents

4.1.2.1. Gypsy Moth – The clearest primary effect of gypsy moth infestations is on terrestrial plants, primarily trees. Various instars of the gypsy moth larvae will feed on host trees and can cause extensive defoliation which can kill some of the infested trees. On a larger scale, the extensive defoliation and/or death of trees may result in secondary changes to vegetation, which will, in turn, affect other forms of vegetation as well as various animal species (primarily related to changes in habitat). Gypsy moth larvae appear to have definite food preferences; oak, birch, poplar, and apple trees seem to be their favorite food sources. While both the European and Asian gypsy moth cause similar types of damage (i.e., defoliation), their feeding preferences are somewhat different, with the Asian gypsy moth preferring a wider range of vegetation. Heavy defoliation is much more common among the oaks than among trees that are not particularly favored as food by the gypsy moth. For susceptible oaks, the effects of infestations on tree mortality varies according to the initial condition of the stand and the number of infestations. Generally, gypsy moth infestations result in mortality of less than 15% of total basal area—i.e., mortality of trees involving 15% the total area of the tree trunks near the ground. When heavy defoliation is followed by massive overstory mortality, existing shrub and herb cover increase dramatically due to increases in available light, moisture, and nutrients. Extensive loss of the existing canopy will also favor the growth of tree species that are intolerant to shade and will shift the forest ecosystem towards earlier successional stages.

The only other groups of organisms likely to be affected directly by the gypsy moth are some and probably very few other lepidopteran species, including the northern tiger swallowtail butterfly. The mechanisms for direct adverse effects on other lepidopteran species may include bacterial contamination of the leaves by gypsy moth larvae and a decrease in the nutritional value of the leaves damaged by the gypsy moth. Most studies, however, do not indicate substantial direct effects on other insects, including *Lepidoptera*. In some cases, increases may be seen in populations of insect predators of the gypsy moth.

There is no evidence in the literature of direct adverse effects of the gypsy moth on most groups of animals. Indirect effects, associated with damage to vegetation, may be of substantial consequence to some species, including squirrels, mice, and other mammals that rely on acorns. Although some mammals consume insects, including the gypsy moth, there is no evidence that gypsy moth outbreaks have a substantial impact on insectivorous mammals. Similarly, birds and aquatic species are not likely to be affected directly or adversely by the gypsy moth. In some species of birds, gypsy moth infestations and subsequent defoliation may be beneficial, especially for those species favoring dead wood as a habitat.

4.1.2.2. B.t.k. – The hazard identification for mammals is closely related to the hazard identification for the human health risk assessment in that both are based, in part, on numerous

standard toxicity studies in experimental mammals. Although *B.t.k.* may persist in mammals for several weeks after exposure, there is little indication that oral or dermal exposure leads to serious adverse effects. Most inhalation studies do not suggest a potential for adverse effects even at *B.t.k.* concentrations much greater than those likely to be encountered in the environment. The lack of a positive hazard identification is supported by field studies which demonstrate a lack of adverse effects in populations of mammals after applications of *B.t.k.*

Toxicity studies in birds are limited to standard acute exposures required by U.S. EPA for product registration. The studies all involve either single-dose gavage administration or five daily dose gavage administrations, and none of the studies reports signs of toxicity or pathogenicity at single oral doses up to 3333 mg formulation/kg bw or at multiple oral doses up to 2857 mg formulation/kg bw. Due to the lack of toxicity of *B.t.k.* formulations as well as other *B.t.* strains, the U.S. EPA did not require chronic or reproductive toxicity studies in birds. This apparent lack of toxicity is supported by numerous field studies in birds. In one field study, a transient decrease in abundance was noted in one species, the spotted towhee (*Pipilo maculatus*). This observation is inconsistent with other field studies on *B.t.k.,* and, according to the investigators, may be an artifact of the study design.

The mechanism of action of *B.t.k.* in *Lepidoptera* is relatively well characterized. *B.t.k.* vegetative cells produce spores and crystals. After the insect consumes the crystals, toxins are formed that attach to the lining of the mid-gut of the insect and rupture the cell walls. The *B.t.k.* spores germinating in the intestinal tract enter the body cavity through the perforations made by the crystal toxins and replicate causing septicemia and eventually death. While various strains of *B.t.* are often characterized as selective pesticides, *B.t.k.* is toxic to several species of target and nontarget *Lepidoptera*. Sensitive nontarget *Lepidoptera* include larvae of the Karner blue butterfly, two species of swallowtail butterflies, a promethea moth, the cinnabar moth, and various species of Nymphalidae, Lasiocampidae, and Saturniidae.

While some nontarget lepidopteran species appear to be as sensitive as target species to *B.t.k.*, most studies indicate that effects in other terrestrial insects are likely to be of minor significance. There is relatively little information regarding the toxicity of *B.t.k.* or *B.t.k.* formulations to terrestrial invertebrates other than insects. Some oil-based *B.t.k.* formulations may be toxic to some soil invertebrates; however, the toxicity is attributable to the oil in the formulation and not to *B.t.k.* There is no indication that *B.t.k.* adversely affects terrestrial plants or soil microorganisms.

The U.S. EPA classifies *B.t.k.* as virtually non-toxic to fish, and this assessment is consistent with the bulk of experimental studies reporting few adverse effects in fish exposed to *B.t.k.* concentrations that exceed environmental concentrations associated with the use of *B.t.k.* in USDA programs. Although there are no data regarding the toxicity of *B.t.k.* or its formulations to amphibians, other strains of *B.t.* appear to have low toxicity to amphibians. The effects of *B.t.k.* on aquatic invertebrates is examined in standard laboratory studies and in numerous field studies. At concentrations high enough to cause decreases in dissolved oxygen or increased biological

oxygen demand, *B.t.k.* may be lethal to certain aquatic invertebrates, like *Daphnia magna*. Most aquatic invertebrates, however, seem relatively tolerant to *B.t.k.* This assessment is supported by several field studies that have failed to note remarkable effects in most species after exposures that substantially exceed expected environmental concentrations. As with effects on terrestrial plants, the toxicity of *B.t.k.* to aquatic plants has not been tested.

The U.S. EPA's Office of Pesticides (U.S. EPA/OPP 1998) has raised concerns that some batches of *B.t.* may contain heat labile exotoxins that are toxic to *Daphnia*. The production of these toxins is an atypical event thought to be associated with abnormal or poorly controlled production processes. The U.S. EPA requires manufacturers to submit a daphnid study on each new manufacturing process to demonstrate that heat labile exotoxin levels are controlled.

4.1.2.2. LdNPV **–** Similar to the hazard identification for the human health risk assessment, the hazard identification for nontarget wildlife species fails to identify any adverse effects of concern—i.e., there is no indication that LdNPV or the Gypchek formulation of LdNPV has the potential to cause adverse effects in any nontarget species. The mammalian toxicity data base for LdNPV is reasonably complete and indicates that LdNPV is not pathogenic or otherwise toxic to mammals. One specific study conducted on wildlife mammals that may consume contaminated gypsy moth larvae indicates no adverse effects in mice, shrews, and opossums. Relative to the large number available studies in mammals, few studies are available in birds but the results of these studies are nearly identical to those in mammals indicating that exposures to LdNPV at levels substantially higher than those likely to occur in the environment will not be associated with adverse effects. Based on bioassays of LdNPV on numerous nontarget insect species and supported by the generally high species specificity of related baculoviruses, the hazard identification for LdNPV in nontarget insects is strikingly similar to that in birds and mammals. There is no indication LdNPV will cause adverse effects in nontarget insects at any level of exposure. Relatively few studies regarding the toxicity of LdNPV have been conducted in fish or aquatic invertebrates; nevertheless, these studies are consistent with studies in terrestrial species, indicating a lack of toxicity to fish and aquatic invertebrates. No data are available on the effects of LdNPV on amphibians, aquatic or terrestrial plants, or other microorganisms. While this lack of information does, by definition, add uncertainty to this risk assessment, there is no basis for asserting that effects on these or other organisms are plausible.

4.1.3. Chemical Agents

4.1.3.1. Diflubenzuron and Tebufenozide **–** The toxicity of diflubenzuron and tebufenozide is well characterized in most groups of animals, including mammals, birds, terrestrial invertebrates, fish, and aquatic invertebrates. In general, both of these compounds are much more toxic to some invertebrates, specifically arthropods, than to vertebrates or other groups of invertebrates.

This differential toxicity of these two compounds involves fundamentally different and well understood mechanisms of action, with tebufenozide being somewhat more selective than diflubenzuron. Toxicity of diflubenzuron to sensitive invertebrate species is based on the inhibition of chitin synthesis. Chitin is a polymer (repeating series of connected chemical

subunits) of a glucose-based molecule and is a major component of the exoskeleton, outer body shell, of all arthropods. The inhibition of the formation of chitin disrupts the normal growth and development of insects and other arthropods. Both terrestrial and aquatic arthropods are affected, but there seems to be some substantial differences in sensitivity. The toxicity of tebufenozide to sensitive invertebrates is based on the mimicking of 20-hydroxyecdysone, an invertebrate hormone that controls molting. The effectiveness of tebufenozide in mimicking 20-hydroxyecdysone activity, however, appears to vary markedly among orders and species of invertebrates. In general, moths are sensitive to tebufenozide but other insects are much less sensitive.

The most sensitive effects in wildlife mammalian species and possibly other vertebrates exposed to diflubenzuron or tebufenozide are likely to be the same as those in experimental mammals (i.e., effects on the blood). The major difference between the hazard identification for diflubenzuron and tebufenozide concerns potential reproductive effects. As noted in the comparative human health risk assessment, tebufenozide may cause reproductive effects in mammals, while this effect has not been noted for diflubenzuron. Similarly, the reproductive effects of tebufenozide but not diflubenzuron are of concern for birds, although the data are somewhat inconsistent. The available studies on tebufenozide include a reproduction study investigating effects in mallard ducks and two reproduction studies investigating effects in bobwhite quail. In one of the quail studies, dietary concentrations of 300 and 1000 ppm caused reproductive effects. The effects were not observed in that study at 100 ppm; moreover, the effects were not observed in the more recent quail study or in the study on mallard ducks. A field study regarding the effects of tebufenozide on reproductive performance in birds noted trends that were not statistically significant but, nonetheless, suggestive of adverse reproductive effects in a warbler species. Thus, consistent with the interpretation by the U.S. EPA, reproductive effects in both mammals and birds are considered endpoints of concern for tebufenozide. For diflubenzuron, there is only one relatively old report of reproductive effects in birds and the effects reported have not been noted in other studies. Thus, also consistent with the approach taken by U.S. EPA, reproductive effects are not identified as an endpoint of concern for diflubenzuron.

Terrestrial invertebrates appear to be much more sensitive to diflubenzuron and tebufenozide than are vertebrates, and tebufenozide appears to affect a narrower group of invertebrates than does diflubenzuron. The terrestrial species most sensitive to diflubenzuron are arthropods, a large group of invertebrates, including insects, crustaceans, spiders, mites, and centipedes. In terrestrial organisms, the species most sensitive to diflubenzuron include lepidopteran and beetle larvae, grasshoppers, and other herbivorous insects. More tolerant species include bees, flies, parasitic wasps, adult beetles, and sucking insects. Tebufenozide is toxic to a much narrower range of terrestrial insects. In general, moths are sensitive to tebufenozide but other insects are much less sensitive.

Both diflubenzuron and tebufenozide are also more toxic to aquatic invertebrates than they are to fish. U.S. EPA has classified diflubenzuron as practically non-toxic to fish, with LC_{50} values that

range from 25 to 500 mg/L. Tebufenozide is somewhat more toxic to fish, with LC_{50} values that range from 2.2 to 6.5 mg/L for fish, categorized as moderately toxic using the U.S. EPA classification system. Invertebrates are affected at much lower concentrations and the relative potency of the two compounds is reversed, with diflubenzuron being substantially more toxic than tebufenozide to aquatic invertebrates. The NOEC values in invertebrates for diflubenzuron are as low as 0.3 µg/L in acute studies and 0.04 µg/L in longer-term studies. Tebufenozide is substantially less toxic to invertebrates, with NOEC values as low as 120 µg/L in acute studies and 3.5 µg/L in longer-term studies.

4.1.3.2. DDVP – Although DDVP is a general neurotoxin, the available data suggest that invertebrates are more sensitive than other organisms to DDVP. For example, the oral LD_{50} in honey bees is 0.29 mg/kg bee, and the topical LD_{50} is 0.65 mg/kg bee. Although DDVP is also toxic to birds, the oral LD_{50} value is about 10 mg/kg for the most sensitive species. Short-term repeat dose studies in mammals found that oral exposures to doses below about 0.5 mg/kg-day or inhalation exposures to 1–2 mg/m³ generally do not result in adverse effects. Thus, no effects are apparent in experimental mammals at doses that are clearly lethal to bees.

Aquatic animals are also sensitive to DDVP. As with terrestrial animals, invertebrates appear to be more sensitive than vertebrates. The lowest reported LC_{50} value in fish is approximately 0.2 mg/L. Some aquatic invertebrates are much more sensitive than fish to DDVP. For daphnids, the most sensitive group of invertebrate species, reported EC_{50} values range from 0.00007 to 0.00028 mg/L.

Most of the toxicity data on ecological receptors is limited to free DDVP, rather than a slow-release formulation like the Vaportape II product used in USDA programs to control the gypsy moth. Hence, the toxicity values reported for indicator species are likely to be conservative (i.e., suggest greater toxicity), as compared with Vaportape II. Although U.S. EPA assessed the ecological effects of DDVP, the exposures assessed are not specific to formulations in which DDVP is encapsulated in PVC resin. In general, aside from those organisms that enter the milk carton trap or those that remove the PVC strip form the trap, toxicity resulting from exposure of ecological receptors to DDVP in Vaportape II milk carton traps is not likely.

4.1.3.3. Disparlure – There is very little information regarding the toxicity of disparlure to nontarget wildlife species. As noted in the human health risk assessment, the U.S. EPA does not require extensive testing of insect pheromones. Thus, the only studies available are acute studies in bobwhite quail, mallard ducks, rainbow trout, bluegill sunfish, *Daphnia magna*, and Eastern oysters. No chronic exposure studies were identified.

Results of acute gavage and dietary exposure studies in mallard ducks and bobwhite quail show that disparlure has very low toxicity in these species, with no mortalities observed following exposure to up to 2510 mg/kg in bobwhite quail. Limited data are available regarding the toxicity disparlure to aquatic animals. Relative to mammals and birds, *Daphnia* appear to the

most sensitive species tested, with an LC_{50} value of 0.098 mg/L. In rainbow trout, 20% mortality was noted at a concentration of 100 mg/L.

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview

Table 4-2 summarizes the exposure assessments on nontarget species for each of the agents covered in the risk assessment. Table 4-2 is similar to the corresponding table for the human health risk assessment (see Table 3-2) because the applications and uses for each control agent are identical. Since diflubenzuron, tebufenozide, LdNPV, and disparlure can be applied in broadcast applications, exposure potential is high and in many cases unavoidable, as is true for the human health risk assessment. When disparlure is used as an attractant in traps, exposures will be variable and primarily incidental. Exposures to the gypsy moth itself are also variable and depend on the extent of the gypsy moth population, which can range from low level infestation to outbreak conditions.

There are, however, notable differences between the human health exposure assessment and the ecological exposure assessment. Unlike Table 3-2, Table 4-2 does not provide measures of exposure for each agent, because the measures of exposure for ecological effects vary not only among the control agents but also among the target groups for each agent. For example, exposures to the gypsy moth are measured as egg masses/acre in the human health risk assessment, which is the same measure of exposure used for terrestrial vegetation, because it is the primary determinant in the dose-response assessment for plants. For all other species, however, effects from the gypsy moth are most likely to be secondary, which means the exposure assessment for these indirectly affected species is based on defoliation—i.e., the result of the dose-response assessment for terrestrial vegetation is used as the exposure assessment for most other groups of organisms.

Other differences in the exposure assessments for nontarget species are mostly superficial. For each of the chemical agents, the mass of the chemical is typically used as the measure of exposure. Depending on the group, the measure of exposure may be expressed as dose (mg agent/kg bw for most terrestrial species), concentration (mg agent/L of water for aquatic species), or simply as application rate (lb agent/acre). This last measure is used primarily when field studies are the basis for the dose-response assessment.

As in the human health risk assessment, different measures of exposure are used for each of the biological agents. For *B.t.k.*, most of the exposures are characterized simply as an application rate in units of BIU/acre. Nevertheless, colony forming units are used for some of the mammalian exposure scenarios. Also as in the human health risk assessment, no clear hazard potential is identified for LdNPV. The very few exposure scenarios that are quantified in the ecological risk assessment for LdNPV are based on the mass of the formulation, Gypchek.

The level of detail used in the exposure assessments for the different chemicals reflects differences in the use patterns and the nature of the available toxicity data. Full sets of exposure assessments in several groups of animals are developed for diflubenzuron and tebufenozide. As in the human health risk assessment, the exposure assessment for diflubenzuron is elaborated by

the consideration of 4-chloroaniline and the exposure assessment for tebufenozide is elaborated by the consideration of multiple applications.

Disparlure, which may also be applied in aerial broadcast applications, has a much more restricted set of exposure scenarios on far fewer groups of organisms. This difference is due completely to the sparse toxicity data available on this compound. In other words, while a very elaborate set of exposure scenarios could be prepared, these scenarios would serve little purpose because they could not be combined with a dose-response assessment to characterize risk. The exposure assessment for DDVP is also restricted due to the limited number of plausible exposures, given that DDVP is used only in milk carton traps and minimal exposures for nontarget species are anticipated under ordinary conditions.

4.2.2. Biological Agents

4.2.2.1. Gypsy Moth – As in the human health risk assessment, the exposure metameter is dictated by the data used to formulate the dose-response assessment—i.e., egg mass density is the exposure metameter for terrestrial invertebrates and plants because it is the measure on which the dose-response assessment is based. Egg mass densities ranging from 5 to 5000 egg masses/acre are used to estimate responses in terrestrial plants and invertebrates.

Most wildlife species are not affected directly by exposure to the gypsy moth but are more likely to experience indirect effects due to changes in habitat or other environmental conditions secondary to defoliation. Consequently, the exposure assessment for most wildlife species is almost identical to the dose-response assessment for terrestrial plants which is expressed as defoliation caused by gypsy moth larvae. For this exposure assessment, categories of defoliation are defined as normal background defoliation (<30% defoliation), moderate defoliation (30-60% defoliation), and high or severe defoliation (>60% defoliation).

4.2.2.2. B.t.k. – Based on the hazard identification, exposure assessments are presented for three groups: small mammals, terrestrial insects, and aquatic species. While a number of different exposure scenarios could be developed for terrestrial mammals, the only positive hazard identification for *B.t.k.* involves inhalation exposures. As in the human health risk assessment, inhalation exposures ranging from 100 to 5000 cfu/m^3 are used to assess potential risks of serious adverse effects in terrestrial vertebrates. These concentrations are applied to a 20 g mouse and correspond to inhaled doses of 0.00336-0.168 cfu/mouse. While there is no basis for asserting that any oral and/or dermal exposures are likely to cause adverse effects in terrestrial vertebrates, an extremely conservative exposure assessment is developed for combined oral (water and vegetation) and dermal (direct spray) exposures that yields an estimated maximum dose of about 184 mg/kg body weight. For terrestrial insects, the toxicity values used to assess the consequences of observing effects is given in units of BIU/ha. Consequently, the exposure assessment for this group is simply the range of application rates used in USDA programs —i.e., approximately 49-99 BIU/ha. For aquatic organisms, toxicity data are expressed in several different units such as mg formulation/L, IU/L, and cfu/L. Based on application rates used in USDA programs and conservative assumptions concerning the depth of water over which *B.t.k.*

might be sprayed, concentrations in water would be expected to be at or below 0.24 mg formulation/L. As discussed in the hazard identification, there is no basis for asserting that adverse effects in birds, plants, soil microorganisms, or soil invertebrates other than insects are of plausible concern. Consequently, explicit exposure assessments are not conducted for those groups.

4.2.2.3. LdNPV – Numerous wildlife species might be exposed to Gypchek or LdNPV as a result of ground and aerial applications of the Gypchek formulation. The need for any formal risk assessment is questionable, however, because neither Gypchek nor LdNPV appear to cause systemic adverse effects. Nonetheless, to provide some basis for comparing the potential risks of Gypchek with other agents used to control the gypsy moth, two extreme exposure assessments are developed: one for a terrestrial herbivore consuming contaminated vegetation and the other for aquatic organisms in a small pond directly sprayed with Gypchek at the highest application rate. For the terrestrial herbivore, the dose estimates range from 1.1 to 3.2 mg Gypchek /kg bw. For aquatic organisms, concentrations are expressed in units of PIB/L because this unit is used in the corresponding toxicity studies. For a small pond directly sprayed with Gypchek at the highest application rate, the estimated initial concentration is 2.5×10^5 PIB/L. Several less extreme exposure assessments could be developed but they would not alter the risk assessment given that the extreme exposure assessments are substantially below any level of concern.

4.2.3. Chemical Agents

4.2.3.1. Diflubenzuron and Tebufenozide – As in the human health risk assessment, the exposure assessments for diflubenzuron and tebufenozide are similar. The same set of exposure scenarios are used with the same set of potential target species. The difference in their application rates dominates the quantitative difference in projected exposure to these two chemicals: a single application rate of 0.0625 lb/acre for diflubenzuron and one or two applications at 0.12 lb/acre for tebufenozide. As a result of the higher application rate for tebufenozide, all exposures are higher for tebufenozide than for diflubenzuron. Also as in the human health risk assessment, the exposure assessments for diflubenzuron are elaborated to include 4-chloroaniline as an environmental metabolite of diflubenzuron.

Not withstanding the quantitative differences in the application rates, the patterns of exposure for terrestrial species for diflubenzuron and tebufenozide are similar except for the maximum acute exposure. For diflubenzuron, this exposure is associated with direct spray of a small mammal and could reach 10 mg/kg. For tebufenozide, the maximum acute exposure is associated with a fish-eating bird and could be as high as 85 mg/kg. For other acute and longer-term exposures, the consumption of contaminated vegetation results in higher levels of exposure to both compounds than does the consumption of contaminated water. Estimates of longer-term daily doses for a small mammal consuming contaminated vegetation at the application site range up to 0.005 mg/kg for diflubenzuron and 0.08 mg/kg/day for tebufenozide. The consumption of contaminated water by a small mammal results in estimated doses of up to 0.00001 mg/kg/day for diflubenzuron and 0.0002 mg/kg/day for tebufenozide. Exposures of terrestrial organisms to 4-chloroaniline as a degradation product of diflubenzuron tend to be much lower than the doses

for diflubenzuron. The highest acute exposure to 4-chloroaniline is about 0.2 mg/kg, the approximate dose for the consumption of contaminated water by a small mammal and the consumption of contaminated fish by a predatory bird. The highest longer term exposure to 4-chloroaniline is 0.0002 mg/kg/day, the dose associated with the consumption of contaminated vegetation by a large bird.

As dicussed in Section 4.3, the toxicity data on terrestrial invertebrates are much more extensive for diflubenzuron than tebufenozide, which is directly related to differences in the numbers of field studies available on diflubenzuron (many), compared with tebufenozide (very few). The difference reflects the long-time, extensive use of diflubenzuron, compared with tebufenozide, which is a more recently introduced insecticide. For both chemicals, exposure of terrestrial invertebrates is generally expressed as an application rate from a field study, and no formal exposure assessment is given.

Exposures of aquatic organisms to diflubenzuron or tebufenozide are based essentially on the same information used to assess the exposures of terrestrial species from contaminated water. At the maximum application rates, the upper range of the expected peak concentration in surface water is estimated at 16 µg/L for diflubenzuron and 40 µg/L for tebufenozide.

4.2.3.2. Disparlure – Given the apparent low acute toxicity of disparlure and the lack of any chronic toxicity data, an exposure assessment for terrestrial species would not add to the assessment of risk. Acute exposure studies in *Daphnia* and rainbow trout show that aquatic animals appear more sensitive than terrestrial animals to disparlure. Therefore, an exposure assessment for aquatic species is made based on aerial spray of a pond at an application rate of 29.1 g a.i./acre, with an estimated concentration in pond water of 0.0072 mg a.i./L.

4.2.3.3. DDVP – As in the human health risk assessment, exposure of terrestrial mammals to DDVP from the VaporTape strips used in milk carton traps is likely to be negligible under most circumstances. Nonetheless, it is conceivable that some mammals such as racoons or bears could easily access and tamper with the milk carton trap. Depending on the proportion of the DDVP strip that is consumed, doses (as DDVP in the PVC strip) are estimated to range from 10.5 mg/kg (10% of strip) to 105 mg/kg (100% of strip) and the central estimate is taken as 31.6 mg/kg (30% of strip). In addition, contamination of water with a pest strip is plausible, although probably rare, and is considered in a manner similar to the corresponding scenario in the human health risk assessment (see Section 3.2.3.4). This scenario is based on the consumption of contaminated water by a small mammal, and the dose to the animal is estimated at about 0.00003 mg/kg with a range from 0.000009 to 0.00009 mg/kg. Other exposure scenarios for terrestrial vertebrates, while possible, seem far less plausible and are not considered quantitatively. No quantitative exposure assessments for terrestrial invertebrates are developed because the milk carton trap will attract only male gypsy moths. Nontarget insects that incidentally enter the trap are likely to be killed by exposure to the DDVP vapor. Exposures to aquatic species are based on the same water concentrations used for terrestrial species: 0.000177 mg/L with a range from 0.000059 to 0.00059 mg/L.

4.3.3. DOSE-RESPONSE ASSESSMENT

4.3.3.1. Overview

An overview of the dose-response assessment for groups of nontarget species is presented in Table 4-3. The information in this table categorizes the data descriptively rather than in terms of data quality. The categories reflect whether the data are sufficient to quantify risk or quantitatively characterize differences in sensitivity among several species in the designated group (●), whether the dose-response assessment is based on both an effect and no-effect level (■), whether the dose-response assessment is based only on a no-effect level (□), or whether the assessment is based only on an effect level (○). These categories are reasonable measures of data quality for all of the agents covered in this risk assessment except LdNPV.

All of the risk values for LdNPV are based on no- effect concentrations or doses. In general, confidence in any dose-response relationship is enhanced if a clear dose-response relationship can be demonstrated and both effect and no-effect exposures have been identified. In the case of LdNPV, however, there is simply no indication that LdNPV or the Gypchek formulation will cause toxicity in any nontarget species at any dose level. While additional studies could be conducted at higher doses and while these studies would enhance confidence in the risk assessment, the NOAEL and NOEC values that have been identified are far above any plausible exposures. Thus, while based on limited data in terms of the dose-effect characterization, the dose-response assessment for LdNPV is adequate for risk characterization.

For most of the other agents, the dose-response assessments are reasonably good for the species of greatest concern. As noted in Table 4-3, dose-response assessments for DDVP are derived only for mammals, fish, and aquatic invertebrates. As discussed in the exposure assessment, this limited approach is taken with DDVP because of the limited use of DDVP in programs to control the gypsy moth. The DDVP is contained in a PVC strip that is placed in a milk carton trap that includes disparlure as an attractant for the gypsy moth. This type of use limits potential exposure for most nontarget species. A formal dose-response assessment is not conducted for terrestrial invertebrates. This is not due to any lack of data. The toxicity of DDVP to insects and many other invertebrates is very well characterized. DDVP is such a potent insecticide that no formal dose-response assessment is needed. Insects and many other species that enter the trap are likely to be killed by exposure to DDVP.

Disparlure is the other agent for which a full set of dose-response assessments are not conducted. As discussed in the hazard identification, this is due to the limited amount of data regarding the toxicity of disparlure to nontarget species.

Relatively full dose-response assessments on groups of greatest concern are given for the gypsy moth, *B.t.k.*, diflubenzuron and its 4-chloroaniline metabolite, and tebufenozide. For the gypsy moth, the effect of primary concern is damage to vegetation. While data are available on both lethality in trees as well as defoliation, defoliation is used as the sublethal effect of primary concern. A dose-response assessment is also given for nontarget lepidopterans. While effect and

no-effect levels can be identified, the significance of this effect is questionable. In terms of direct effects, terrestrial vegetation is the primary target of concern.

Lepidoptera are the primary nontarget group of concern for *B.t.k.* exposure. A relatively rich set of studies is available regarding the sensitivities of nontarget *Lepidoptera* and some other insects. The sensitivities of the nontarget insects can be quantified reasonably well from exposures that encompass the application rates used in USDA programs to control the gypsy moth. Sensitive nontarget *Lepidoptera* include larvae of the endangered Karner blue butterfly as well as several other types of moths.

Similar types of information are available on diflubenzuron and tebufenozide, and dose-response assessments can be made for the species of primary concern. For both chemicals, this includes nontarget *Lepidoptera* and aquatic invertebrates. Other terrestrial arthropods are also considered for diflubenzuron. In addition, because of the standard tests required by U.S. EPA for the registration of most pesticides, adequate toxicity data are available on mammals, birds, and fish. The toxicity data base for diflubenzuron is somewhat more extensive and sensitivities in nontarget organisms are somewhat better defined in both laboratory and field studies than is the case with tebufenozide.

4.3.2. Biological Agents

4.3.2.1. Gypsy Moth – As in the human health risk assessment for the gypsy moth, the dose measure for the gypsy moth is egg masses/acre. Quantitative dose-response assessments can be made for both terrestrial plants and sensitive species of *Lepidoptera*. The dose-response assessments for terrestrial plants are based on a relatively simple quantitative model for the relationship of egg mass density and vegetation type to defoliation. Three broad categories of vegetation (sensitive, intermediate, and tolerant) are used to characterize the susceptibility of forest stands to gypsy moth induced defoliation. Estimated LOAEL values based on 30% defoliation, which is considered the lower range of moderate defoliation, are approximately 125 egg masses/acre for sensitive stands, 1000 egg masses/acre for intermediate stands, and 7000 egg masses/acre for tolerant stands. The corresponding NOAEL values, defined as 10% defoliation, are estimated as 12, 20, and 125 egg masses/acre for sensitive, intermediate, and tolerant forest stands.

The effects of gypsy moth exposure on sensitive terrestrial invertebrates, including some species of *Lepidoptera*, are less well documented and less well characterized, compared with the effects on terrestrial plants. Nonetheless, available studies indicate that the NOAEL for adverse effects in certain other species of *Lepidoptera* are lower than the NOAEL for sensitive forest stands—— i.e., about 6-72 egg masses/acre for some *Lepidoptera*. No quantitative dose-response assessment is presented for other groups of organisms—e.g., mammals, birds, and soil or aquatic organisms. The impact of gypsy moth exposure on these species is most likely to result in indirect effects secondary to defoliation. This is discussed further in the risk characterization.

4.3.2.2. B.t.k. – As summarized in Table 4-3, exposure assessments are presented for four groups: mammals, terrestrial insects, fish, and invertebrates. While a number of different exposure scenarios could be developed for terrestrial mammals, the only positive hazard identification for *B.t.k.* involves inhalation exposures. As in the human health risk assessment, inhalation exposures of 100-5000 cfu/m^3 are used to assess potential risks of serious adverse effects in terrestrial vertebrates. These concentrations are applied to a 20 g mouse and correspond to inhaled doses of 0.00336-0.168 cfu/mouse. While there is no basis for asserting that any oral and/or dermal exposures are likely to cause adverse effects in terrestrial vertebrates, an extremely conservative exposure assessment is developed for combined oral (water and vegetation) and dermal (direct spray) exposures that yields an estimated maximum dose of approximately 184 mg/kg body weight.

For terrestrial insects, the toxicity values used to assess the consequences of observing effects is given in units of BIU/ha over a range of applications similar to those used in gypsy moth control programs. The magnitude of response to *B.t.k.* in sensitive nontarget species appears similar to that of the gypsy moth. Tolerant species appear to be about 30-fold less sensitive than the gypsy moth to B.t.k.. The designations of sensitive and tolerant species are not intended to imply absolute ranges on tolerance among all possible insects. Instead, the dose-response assessments for this group simply indicate that some nontarget species, such as the Karner blue butterfly and cinnabar moth, appear to be as sensitive to *B.t.k.* as target species such as the gypsy moth and cabbage looper. The range of sensitivities among various insect species appears to follow a continuum, and it is possible that some species may be more or less sensitive to *B.t.k.* than those insects on which toxicity data are available.

For aquatic organisms, toxicity data are expressed in several different units such as mg formulation/L, IU/L, and cfu/L. Based on application rates used in USDA programs and conservative assumptions concerning the depth of water over which *B.t.k.* might be sprayed, concentrations in water would be expected to be at or below 0.24 mg formulation/L. Toxicity values for fish are 1.4 mg formulation/L (an LOEC for sensitive species) and 1000 mg formulation/L (an NOEC for tolerant species). For aquatic invertebrates, the NOEC values for sensitive and tolerant species are 0.45 and 36 mg/L, respectively.

4.3.2.3. LdNPV – Because no hazards can be identified for any species, a quantitative dose-response assessment is not required. Consequently, no dose-response assessments were proposed by U.S. EPA and none were used in the previous gypsy moth risk assessment for Gypchek. In order to provide a quantitative comparison of the risks of using Gypchek relative to the other agents, dose-response assessments are made for both terrestrial mammals and aquatic species. For terrestrial mammals, the NOAEL of 2600 mg/kg bw is used. This is the same NOAEL that serves as the basis for the surrogate acute RfD for LdNPV in the human health risk assessment for this agent. For aquatic species, only NOEC values are available, and the highest NOEC of $8x10^9$ PIB/L is used to characterize risk.

4.3.3. Chemical Agents

4.3.3.1. Diflubenzuron and Tebufenozide – As summarized in Table 4-3, the dose-response assessments for diflubenzuron and tebufenozide are far more complete, in terms of the number of groups encompassed, than are the corresponding assessments for other agents considered in this risk assessment. This difference reflects both the nature of the available data and an assessment of the need to characterize risk quantitatively. Despite their specific modes of action in target species, diflubenzuron and tebufenozide induce toxicological responses in many different groups of animals. Furthermore, both chemicals are used in broadcast aerial applications, making exposure to many different groups of organisms likely.

Both diflubenzuron and tebufenozide are relatively non-toxic to mammals and birds. As noted in the human health risk assessment, the acute and chronic toxicities of these two chemicals in mammals appear to be virtually identical in terms of NOAELs. This is also true for birds. The toxicity values used in the ecological risk assessment for mammals are identical to those used in the human health risk assessments: an acute NOAEL of 1118 mg/kg and a chronic NOAEL of 2 mg/kg/day for diflubenzuron and an acute NOAEL of 1000 mg/kg and a chronic NOAEL of 1.8 mg/kg/day for tebufenozide. The differences between the values for the chemicals are clearly insubstantial. For birds, the acute NOAEL for diflubenzuron is taken as 2500 mg/kg and the longer-term NOAEL is taken as 110 mg/kg/day. For tebufenozide, the values are again very similar: an acute NOAEL of 2150 mg/kg and a longer-term NOAEL of 15 mg/kg/day. For both chemicals, the longer-term NOAEL is taken from standard assays on reproduction.

In terms of potential effects on terrestrial invertebrates, the data set for diflubenzuron is much richer than the data set for tebufenozide. Many laboratory toxicity studies and field studies have been conducted on diflubenzuron. Field studies are used in the dose-response assessment of diflubenzuron because the standard toxicity studies are extremely diverse and many are not directly applicable to a risk assessment. Despite the difficulty and uncertainty in interpreting some of the field studies, the relatively large number of field studies on diflubenzuron appears to present a reasonably coherent pattern that is at least qualitatively consistent with the available toxicity data and probably a more realistic basis on which to assess risk to nontarget species. The most sensitive species appear to be grasshoppers which may be adversely affected at an application rate of about 0.02 lb/acre [22 g/ha]. Somewhat high application rates—in the range of 0.027-0.031 lb/acre [30 to 35 g/ha]—will adversely affect macrolepidoptera and some beneficial parasitic wasps. At the maximum application rate considered in this risk assessment—0.062 lb/acre [70 g/ha]—some additional herbivorous insects are likely to be affected. No adverse effects in several other groups of insects are expected at this or much higher application rates. Honeybees are among the most tolerant species and are not likely to be adversely affected at application rates of up to 0.35 lb/acre [400 g/ha]. Invertebrates that do not synthesize chitin are also relatively tolerant to diflubenzuron.

Although there are fewer and generally less detailed field studies on tebufenozide, compared with diflubenzuron, it appears to be less toxic to nontarget species (e.g., lacewing). In general, the field studies indicate that tolerant insect species are not affected by tebufenozide at application

rates up to 0.24 lb/acre. The true NOEC may be higher – i.e., an LOEC has not been identified for tolerant species of terrestrial insects. Conversely, application rates as low as 0.03 lb/acre have been shown to have adverse effects on sensitive nontarget insects, primarily *Lepidoptera*. A NOEC for sensitive species was not identified.

For both diflubenzuron and tebufenozide, the toxicity values for aquatic species follow a pattern similar to that for terrestrial species: arthropods appear to be much more sensitive than fish or non-arthropod invertebrates. Both compounds are about equally toxic to fish with virtually identical chronic NOEC values: 0.05 mg/L for diflubenzuron and 0.048 mg/L for tebufenozide.

There are major and substantial differences regarding the toxicity of diflubenzuron and tebufenozide to aquatic invertebrates. Diflubenzuron is much more toxic. In acute toxicity studies, the NOEC for the most sensitive species is 0.0003 mg/L diflubenzuron, which is 400 times less than the corresponding NOEC of 0.12 mg/L for tebufenozide. Chronic toxicity studies indicate a similar pattern. The NOEC for the most sensitive species is 0.00004 mg/L for diflubenzuron and 0.0035 mg/L for tebufenozide. The difference is a factor of about 90 [0.0035 mg/L / 0.00004 mg/L]. Even though the number of available NOEC values is greater for diflubenzuron (seven acute and seven chronic), compared with tebufenozide (three acute and two chronic), and variability can be expected to increase as the number of species tested increases, it is unlikely that the apparent differences in toxicity are artifacts of sample size. For example, based on acute and chronic NOEC values in *Daphnia*, which are available for both compounds, diflubenzuron is more toxic than tebufenozide by a factor of about 2700 in acute studies and a factor of 725 in chronic studies. The toxicity to aquatic invertebrates is one of the few areas in which diflubenzuron and tebufenozide differ remarkably, and this difference has an impact on the risk characterization (Section 4.4).

4.3.3.2. Disparlure – The limited amount of toxicity data on disparlure precludes making a standard dose-response assessment for terrestrial species. Disparlure is identical or similar to pheromones produced by other species of moths and is able to attract male nun moths. Since, however, there are no quantitative data available regarding the efficacy of disparlure in nontarget moths, a dose-response assessment for this effect in a nontarget species of moths cannot be made. For aquatic species, NOEC values and limited data on effect levels are available from acute exposure studies in rainbow trout and *Daphnia*. No LC_{50} values are available in fish. The dose-response assessment is limited to NOEC values of 10 mg/L in trout and 300 mg/L in bluegills. The only information on toxic effects in fish consists of a report of 20% mortality in trout after acute exposure to disparlure at 100 mg/L. Thus, disparlure does not appear to be highly toxic to fish. *Daphnia magna* are much more sensitive with a 48-hour LC_{50} of 0.098 mg/L and an NOEC for mortality of 0.017 mg/L. Based on the LC_{50} value, disparlure is classified as highly toxic to aquatic invertebrates.

4.3.3.3. DDVP – Given the limited nature of the use of DDVP in programs to control the gypsy moth and consequent limited number of exposure assessments, the dose-response assessment for DDVP is relatively simple. For terrestrial mammals, a value of 240 mg/kg from a study using

DDVP in a PVC formulation is used for direct exposure to the DDVP-PVC strip—i.e., a raccoon tampering with a milk carton trap and consuming all or part of the DDVP strip. At the dose of 240 mg/kg, no mortality or frank signs of AChE inhibition were observed. For the contaminated water scenario, the NOAEL of 0.5 mg/kg from a study involving exposure to free or unformulated DDVP is used. This NOAEL is from the study that forms the basis for the acute RfD used in the human health risk assessment. Although DDVP is classified as highly toxic to fish, the estimated levels of acute exposure for fish are far below the 30-day NOEC of 0.03 mg/L. Thus, this value is used for all fish and no attempt is made to consider differences in sensitivity among fish. A somewhat different approach is taken with aquatic invertebrates, some of which are more sensitive than fish to DDVP by a factor of more than 2500. Risks to sensitive species of aquatic invertebrates —i.e., daphnids and other small arthropods—are characterized based on the lowest reported LC_{50} value, 0.00007 mg/L from a 48-hour bioassay in *Daphnia pulex*. Some other groups of aquatic invertebrates, such as snails, appear to be much less sensitive than small arthropods. Risks to such tolerant species are based on a LC_{50} value of 21 mg/L in a freshwater snail.

4.4. RISK CHARACTERIZATION

4.4.1. Overview

The comparative risk characterization for the ecological risk assessment is expressed similarly to that in the human health risk assessment. Numerically, the risk characterizations are given as hazard quotients (HQs), the level of exposure divided by some measure of effect, typically an NOAEL or NOEC. As in the human health risk assessment, the comparative risk characterization for ecological effects typically categorizes concern with the agents as marked (HQ>10), marginal (HQs between about 0.1 and 10), and minimal (HQ<0.1). One exception is made for *B.t.k.*, which is classified as an agent of marked concern although the highest HQ is 9.4.

An overview of the comparative risk characterization is summarized in Table 4-4 for terrestrial species and Table 4-5 for aquatic species. The risk characterizations are illustrated in Figure 4-1 (terrestrial) and Figure 4-2 (aquatic). As in the human health risk assessment, the HQs for each agent are presented as a range. The upper end of the range is typically the highest hazard quotient associated with a plausible exposure scenario. The lower end of the range is not necessarily the lowest HQ calculated in each of the risk assessments. For some agents, the lower range is taken from sets of exposure scenarios that provide similar HQs for exposures that may be regarded as typical. For these agents, the lowest HQs reported in the individual risk assessments are close to zero. In some cases, the numerical expressions of risk do not adequately convey the potential for hazard. These cases are noted in Figures 4-1 and 4-2 with comments.

Ecological risk assessments involve, at least implicitly, considerations of thousands of different species and the relationships among these species and their habitats. Invariably, however, data are available on only a small subset of these species and field studies provide only limited insight into the complex interrelationships and secondary effects among species. Thus, as in the human health risk assessments, ecological risk assessments cannot offer a guarantee of safety. They can and do offer a means to identify whether or not there is a basis for asserting that adverse effects are plausible and what the nature of these effects might be.

Within these limitations, only LdNPV clearly qualifies as an agent of minimal concern. While there are limitations in the available studies on LdNPV, there is simply no basis for asserting that LdNPV will adversely affect any species except the gypsy moth.

Agents of marked concern include the gypsy moth, *B.t.k.*, and diflubenzuron. The types of concern with each of these agents, however, are quite different. For both the gypsy moth and *B.t.k.*, the concerns are narrow. The gypsy moth clearly will damage some terrestrial vegetation. *B.t.k.* is likely to affect sensitive *Lepidoptera*. Concern with the use of diflubenzuron is broader and includes effects on both terrestrial and aquatic invertebrates.

The designation of the gypsy moth as an agent of marked concern is obvious. The effects of gypsy moth larvae on forests are extremely well documented and well understood. In sensitive forest stands—i.e., stands in which oak, birch, and other favored species predominate —gypsy moth larvae can cause substantial defoliation and tree mortality. While some other lepidopteran

species also may be directly affected by exposure to the gypsy moth, most of the other effects caused by the gypsy moth will be secondary. Reductions in populations of squirrels, mice, and other mammals which may be sensitive to changes in the availability of acorns are likely and have been well documented. Substantial secondary adverse effects on other groups of animals—i.e., birds, reptiles, and aquatic species—cannot be ruled out but have not been convincingly or consistently demonstrated.

Diflubenzuron is also clearly an agent of marked concern. Exposures to diflubenzuron at application rates used in gypsy moth control programs will adversely affect both terrestrial and aquatic invertebrates that rely on chitin for their exoskeleton. This has been demonstrated in controlled toxicity studies as well as multiple field studies.

The designation of *B.t.k.* as an agent of marked concern is somewhat judgmental. As noted in Table 4-4, the highest hazard quotient is 9.4. Based on this HQ and the classification scheme used generally, *B.t.k.* would be classified as an agent of marginal concern. However, recent studies convincingly demonstrate that adverse effects in nontarget *Lepidoptera* will occur in the applications of *B.t.k.* used to control the gypsy moth. Concern is heightened because some of the *Lepidoptera* that may be adversely affected include at least one endangered species.

Tebufenozide, DDVP, and disparlure are all classified as agents of marginal concern. For tebufenozide, the numerical expressions of risk may be less relevant than a more qualitative assessment. The highest HQ is 4 and is associated with the consumption of contaminated vegetation by a large mammal after two applications of the compound at the highest labeled application rate. While this exposure would be considered undesirable, it is not clear that any frank signs of toxicity would be seen. Risks to nontarget *Lepidoptera* may be of greater concern but the available data are insufficient to quantify potential risk. Risks to other invertebrates, both terrestrial and aquatic, appear to be insubstantial. DDVP is of marginal concern in that highly localized effects may be expected: nontarget insects entering a milk carton trap or some aquatic invertebrates affected by the accidental contamination of a small body of water with a pest strip. In both cases, the effects would be relatively minor, in terms of the number of organisms affected. Marginal concern for disparlure is associated with the relatively high toxicity of this agent to *Daphnia* and is reenforced by the very scant data on the toxicity of an agent that may be applied to large areas in broadcast applications.

4.4.2. Agents of Marked Concern

4.4.2.1. Gypsy Moth – The best documented and most obvious effect of the gypsy moth will be on terrestrial vegetation, particularly forest stands in which sensitive species of trees predominate. In some respects, the risk characterization for terrestrial vegetation is essentially a restatement of the hazard identification. In other words, the effects of gypsy moth larvae on forests is extremely well documented and relatively well understood. In sensitive forest stands—i.e., stands in which oak, birch, and other favored species predominate —gypsy moth larvae can cause substantial defoliation. In forest stands in which tree species that are not favored by gypsy moth larvae predominate —e.g., hemlock, various types of pine, black locust

and white ash—even relatively high exposures, as measured by egg mass density, may not result in substantial defoliation. The risk assessment for direct effects on forests should be at least qualitatively influenced by the current range of the gypsy moth, which has not yet extended to some forests in the southeast that may be among the most sensitive to gypsy moth exposure. Thus, unless measures to contain the gypsy moth are successful, the southeastern oak forests may suffer serious damage in future infestations.

Some other lepidopteran species also may be directly affected by exposure to the gypsy moth. Most studies, however, suggest that substantial adverse effects in terrestrial insects are unlikely and effects in some insect species, including some other *Lepidoptera*, may be beneficial.

Because the gypsy moth may substantially damage some forests in severe infestations or outbreaks, secondary effects in other species of wildlife are plausible. Reductions in populations of squirrels, mice, and other mammals which may be sensitive to changes in the availability of acorns are likely. Substantial adverse effects on other groups of animals—i.e., birds, reptiles, and aquatic species—cannot be ruled out but have not been convincingly demonstrated.

4.4.2.2. B.t.k. – Terrestrial insects are the only organisms likely to be adversely affected by exposure to *B.t.k.* or its formulations. Separate dose-response curves can be generated for both sensitive and tolerant terrestrial insects. At the application rates used to control gypsy moth populations, mortality rates among sensitive terrestrial insects are likely to range from approximately 80% to 94% or more. All sensitive terrestrial insects are *Lepidoptera* and include some species of butterfly, like the endangered Karner blue and some swallowtail butterflies and promethea moths.

The effects in sensitive species have been convincingly demonstrated in the study by Herms et al. (1997). In this study, the toxicity of Foray 48B was assayed in larvae of both the gypsy moth and the Karner blue butterfly, an endangered species of butterfly indigenous to the northern United States (Minnesota to New Hampshire). Bioassays in both species involved applications of Foray 48B to vegetation (wild lupine leaves for the Karner blue and white oak leaves for the gypsy moth) at treatment levels equivalent to either 30-37 BIU/ha per ha (low dose) or 90 BIU/ha (high dose). A negative control consisted of untreated vegetation. The insect larvae (either 1st or 2nd instar for the Karner blue and 2nd instar for the gypsy moth) were placed on the vegetation 7 to 8 hours after treatment and allowed to feed for 7 days. Survival rates for Karner blue larvae were: 100% for controls, 27% at the 30-37 BIU/ha treatment rate, and 14% at the 90 BIU/ha treatment rate. Survival rates for gypsy moth larvae were: 80% for controls; 33% for low-dose treatment, and 5% for high-dose treatment. Based on a statistical analyses of these data, the gypsy moth and Karner blue appear to be equally sensitive to *B.t.k.* This study is supplemented by the series of bioassays conducted by Peacock et al. (1998) which suggest that various other lepidopteran species may be as sensitive as the gypsy moth to *B.t.k.*.

For some *Lepidoptera*, sensitivity to *B.t.k.* is highly dependent on developmental stage. This is particularly evident for the cinnabar moth, where late instar larvae are very sensitive to *B.t.k.* and

early instar larvae are very tolerant to *B.t.k*. Given the mode of action of *B.t.k.*—i.e., it must be ingested to be highly toxic to the organism— effects on even the most sensitive species will occur only if exposure coincides with a sensitive larval stage of development. In tolerant species, including non-lepidopteran insects and certain larval stages of some *Lepidoptera*, the anticipated mortality rates are much lower (on the order of less than 1% to about 4%).

The risk characterization for terrestrial mammals is unambiguous: under foreseeable conditions of exposure, adverse effects are unlikely to be observed. Similarly, based on a very conservative exposure assessment for aquatic species, effects in fish and aquatic invertebrates appear to be unlikely. As discussed in the hazard identification, effects in birds, plants, soil microorganisms, or soil invertebrates other than insects are not of plausible concern. Thus, quantitative risk characterizations for these groups are not conducted. For oil-based formulations of *B.t.k.* (or any other pesticide), effects in some soil invertebrates are plausible.

4.4.2.3. Diflubenzuron – While the data base supporting the risk assessment of diflubenzuron is large and somewhat complex, the risk characterization is relatively simple and unequivocal. Diflubenzuron is an effective and general insecticide. Application rates used to control the gypsy moth are likely to have effects on some nontarget terrestrial insects. Species at greatest risk include grasshoppers, various macrolepidoptera (including the gypsy moth), other herbivorous insects, and some beneficial predators to the gypsy moth. These species are at risk because of the mode of action of diflubenzuron (i.e., inhibition of chitin) and the behavior of the sensitive insects (the consumption of contaminated vegetation or predation on the gypsy moth). Some aquatic invertebrates may also be at risk but the risks appear to be less than risks to terrestrial insects. The risk characterization for aquatic invertebrates is highly dependant on site-specific conditions. If diflubenzuron is applied when drift or direct deposition in water is not controlled well or in areas where soil losses from runoff and sediment to water are likely to occur, certain aquatic invertebrates are at risk of acute adverse effects, and exposure could cause longer-term effects on more sensitive species.

Direct effects of diflubenzuron on other groups of organisms—that is, mammals, birds, amphibians, fish, terrestrial and aquatic plants, microorganisms, and non-arthropod invertebrates—do not appear to be plausible. Nontarget species that consume the gypsy moth or other invertebrates adversely affected by diflubenzuron may be at risk of secondary effects (for example, a change in the availability of insect prey). There is no indication that 4-chloroaniline formed from the degradation of diflubenzuron will have an adverse effect on any terrestrial or aquatic species.

4.4.3. Agents of Marginal Concern

4.4.3.1. Tebufenozide – The use of tebufenozide to control the gypsy moth may result in adverse effects in nontarget *Lepidoptera* but these effects have not been well characterized or clearly demonstrated. There is little indication that other species will be impacted under normal conditions of use even at the highest application rate. Tebufenozide is an insecticide that is effective in controlling populations of lepidopteran pests. No data, however, are available on

toxicity to nontarget *Lepidoptera*. For the risk assessment of this compound, the assumption is made that nontarget *Lepidoptera* may be as sensitive as target *Lepidoptera* to tebufenozide. Thus, adverse effects in nontarget *Lepidoptera* would be expected after applications that are effective for the control of lepidopteran pest species.

There is no indication that short-term exposures to tebufenozide will cause direct adverse effects in any terrestrial vertebrates or non-lepidopteran invertebrates even at the upper range of plausible exposures or as a result of accidental exposures. Similarly, direct adverse effects from longer-term exposures in birds and mammals appear to be unlikely under most conditions. Effects on birds due to a decrease in available prey—i.e., terrestrial invertebrates—are considered plausible. In extreme cases, exposure levels in some large mammals might exceed the NOEC, but would remain below levels associated with frank signs of toxicity. This point is reflected in the HQ of 4 for a large mammal consuming contaminated vegetation after two applications of tebufenozide at the highest labeled rate. Under normal conditions of use, tebufenozide is not likely to cause adverse effects in aquatic species; however, in the case of a large accidental spill into a relatively small body of water, adverse effects might be expected in aquatic vertebrates, invertebrates, and plants.

4.4.3.2. DDVP – As in the human health risk assessment of DDVP, typical exposures and consequent risks to nontarget species should be negligible. The containment of the DDVP within a slow-release PVC strip combined with the target specific nature of pheromone baited traps should reduce the risks of inadvertent effects on nontarget species. Other insects and arthropods that may inadvertently enter the trap will probably be killed by DDVP vapor. While such inadvertent contact may occur, it is not likely to have a substantial impact on the number of nontarget insects or arthropods.

Because of the limited use of DDVP, a relatively small number of exposure scenarios —all of which might be considered accidental or incidental—are developed. For terrestrial mammals, contact with the pest strip could occur by an animal directly tampering with a trap or by an animal consuming water accidentally contaminated with a DDVP strip. Adverse effects would not be expected in either case. In the case of accidental contamination of a small body of water with a DDVP strip, concentrations of DDVP in the water would be below the level of concern for fish by factors ranging from about 50 to 500. Some aquatic invertebrates, however, might be affected. For the most sensitive species of aquatic invertebrates —i.e., small aquatic arthropods like daphnids—exposure levels could substantially exceed laboratory LC_{50} values by factors of up to about 8. Exposures to tolerant aquatic invertebrates—like snails—would be below a level of concern by a substantial margin—i.e., factors ranging from about 30,000 to 300,000.

The exposure assessments that serve as the bases for these risk characterizations are highly dependent on specific conditions—i.e., how much DDVP was in the strip at the time that the contamination occurred and the size of the body of water that was contaminated. Because the hydrolysis of DDVP in water is rapid, the estimates of adverse effects in some aquatic

invertebrates would probably apply only to a very limited area near the pest strip rather than to the larger area of the body of water that is contaminated.

4.4.3.3. Disparlure – There is little data available on terrestrial and aquatic animals to allow for a quantitative characterization of risk. The lack of chronic toxicity data in any species adds uncertainty to any risk characterization. Thus, for both terrestrial and aquatic species, the potential for the development of toxicity from long-term exposure to disparlure cannot be ruled out. Concern with the lack of toxicity data on disparlure is exacerbated by the fact that this compound may be applied to large areas in broadcast applications.

Nonetheless, based on the available data, clear hazards to nontarget species have not been identified. Disparlure may disrupt mating in some moths other than the gypsy moth. The two species that are known to be affected, however, are both forest pests like the gypsy moth and only one of these other species is native to North America. For aquatic species, hazard quotients for both rainbow trout and *Daphnia* are below one, although the hazard quotient of 0.4 for *Daphnia* approaches one. Thus, while 0.4 is below the level of concern of one, there is uncertainty in the risk characterization because of the limited acute toxicity data, the lack of chronic toxicity data, and the high likelihood that many species will be exposed to this compound.

4.4.4. Agent of Minimal Concern: Gypchek

Unlike all of the other agents considered in this risk assessment, there is no basis for asserting that the use of Gypchek to control or eradicate gypsy moth populations is likely to cause any adverse effects in any species other than the gypsy moth. While no pesticide is tested in all species under all exposure conditions, the data base on LdNPV and related viruses is reasonably complete and LdNPV has been tested adequately for pathogenicity in a relatively large number of species, particularly terrestrial invertebrates. LdNPV appears to be pathogenic and toxic to the gypsy moth and only to the gypsy moth.

For Gypchek, quantitative expressions of risk are in some respects more difficult because clear LOEC values are not defined—i.e., if an agent is not shown to cause an effect, the threshold for effects is not a meaningful concept. Nonetheless, general but very conservative exposure assessments demonstrate that plausible upper ranges of exposures are clearly below any level of concern by a factor of 1000 for terrestrial species and 30,000 for aquatic species.

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Tables

Table 2-1: Total use of control agents by numbers of acres treated between 1995 and 2003

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Table 4-4: Comparative risk characterization for terrestrial species
Table 4-5: Comparative risk characterization for aquatic species

Table 2-1: Total use of control agents by numbers of acres treated between 1995 and 2003

	Suppression (Total acres)	Eradication (Total acres)	Slow-the-Spread (Total acres)	Grand Total (Acres)
B.t.k.	1,484,486	1,057,201	367,722	2,909,409
NPV	36,518	7,376	9,140	53,034
Diflubenzuron	657,671	6	6,883	664,560
Tebufenozide	0	0	0	0
Disparlure flakes	0	60,090	1,567,199	1,627,289
Mass Traps *	0	1,912	0	1,912

* Mass traps contain DDVP in a PVC strip and disparlure as an attractant.

Table 3-1: Comparative hazard identification for potential effects in humans

Endpoint	Agents used in Gypsy Moth Program						
	Gypsy Moth	*B.t.k.*	LdNPV	DFB	Tebufen-ozide	DDVP	Dispar-lure
Lethality	○	□	○	□	□	● [a]	○
Sub-lethal effects							
Irritation	●	●	■	□	○	■	□
Blood				●	●	○ [b]	
Carcinogenicity				■ [c]	○	□	
Neurotoxicity		○	○	□	○	●	
Immunotoxicity		○	○	○	○	□	
Reproduction				○	■	■	
Endocrine Effects					○	□	
Pathogenicity		□ [d]	○	N/A	N/A	N/A	N/A

[a] Risks are mitigated by formulation in PVC.
[b] Excluding inhibition of plasma and RBC AChE.
[c] An environmental metabolite, 4-chloroanaline, poses a carcinogenic risk.
[d] *B.t.k.* itself does not appear to be pathogenic. Possible enhancement of influenza virus.

Key:		
	●	Effect/risk demonstrated in humans
	■	Effect is plausible
	□	Marginal evidence for potential effect
	○	No plausible basis for risk
	Blank	No data are available

Table 3-2: Comparative exposure assessment for human health effects

Agent	Measure of Exposure	Plausibility of Exposure	Comments
Gypsy moth	Eggs masses per acre	Variable	Exposure potential is high during outbreaks and decreases as intensity of infestation decreases.
B.t.k.	Application rate and cfu/m^3 x hour	High	During broadcast applications, exposure potential is high and can be reasonably well characterized.
LdNPV	mass of formulation	High	During broadcast applications, exposure potential is high and can be reasonably well characterized.
Diflubenzuron	mass of chemical	High	Can persist on vegetation and water contamination is plausible.
Tebufenozide	mass of chemical	High	Can persist on vegetation and water contamination is plausible.
DDVP	mass of chemical	Very low	Except in cases of intentional or incidental tampering with a trap, exposures will be very low.
Disparlure	mass of chemical	Variable	Very little compound is used in traps and exposures are likely to be very low.

Table 3-3: Comparative dose-response assessment for human health effects

Agent	Toxicity Value	Endpoint	Quality	Comment
Gypsy Moth	Acute	Irritation	●	Based on human data with a clear dose-response relationship.
B.t.k.	Acute	Irritation	■	Based on human data but no dose-response relationship is apparent.
		Toxicity	□	Based on a single study in mice using a marginally relevant route of exposure.
LdNPV	Acute	None	●	High confidence because no endpoint of concern can be identified.
Diflubenzuron	Acute	Blood	■	No EPA acute RfD. Conservative approach based on petroleum formulation.
	Chronic	Blood	●	Agency-wide EPA RfD adopted by OPP.
4-Chloroaniline*	Acute	Blood	■	No EPA acute RfD. Conservative approach based on 90-day study.
	Chronic	Blood	□	EPA chronic RfD. Confidence classified as low by EPA.
	Cancer Potency	Cancer	■	EPA cancer potency factor
Tebufenozide	Acute	Repro	■	No EPA acute RfD. Based on reproduction studies in two species
	Chronic	Blood	■	EPA/OPP chronic RFD.
DDVP	Acute	Neuro	●/□	For DDVP itself, value is based on an EPA acute RfD. For DDVP in PVC strip, the value is based on marginal data.
Disparlure	Acute	N/A	□	No acute RfD can be derived.

Key for quality of Toxicity Values:

●	High
■	Medium
□	Low

* An environmental metabolite of diflubenzuron.

Table 3-4: Comparative risk characterization for human health effects [a]

Agent	Hazard Quotient (HQ) [b]		Comments
	Lower	Upper	
Gypsy Moth	**1.6**	**625**	Irritant effects (dermal, ocular, and/or respiratory) are well documented. Lower range is based on sparse infestations, where effects might be seen in about 1% of the population. Upper range is based on major outbreaks where responses might be seen in about 40% of the population.
B.t.k.	0	0.04	HQs are for serious adverse effects, which are highly unlikely to occur. Irritant effects could be reported in about 20% of exposed individuals – both workers and members of the general public.
LdNPV	0	0.02	No risks are plausible. Upper range of HQ is calculated from a free-standing NOAEL.
Diflubenzuron			
Workers	0.05	0.5	The upper range is associated with the upper range of plausible exposures in ground spray applications. Under typical conditions, the HQ will be about 0.05.
Public	0.09	0.1	This narrow range of HQs reflects the higher HQ for any longer term exposure (0.09) and the highest HQ for acute exposures (0.1). Most other HQs are below 0.01.
4-Chloroaniline as an environmental metabolite of diflubenzuron			
Toxicity	0.02	0.4	Lower value is based on acute consumption of contaminated water (peak concentration) by child. Upper range based on acute consumption of contaminated fish by subsistence populations after accidental spill. Other HQs are insubstantial.
Cancer	0.09	0.4	HQs based on cancer risk of 1 in 1 million. Both lower and upper are based on consumption of contaminated water (central and upper ranges). Other scenarios lead to much lower risks.
Tebufenozide	0.03	**1.5**	Lower range is based on the central estimate of contaminated fruit (longer-term) after 2 applications. Highest HQ is for the upper range of longer-term consumption of contaminated fruit following 2 applications at the highest application rate. Other HQs are much less than 0.03.
DDVP	0	**380**	Lower range of risk is essentially zero because exposures are unlikely. Upper range is based on oral exposure from a child tampering with the strip. Likelihood of clinically significant effects seems remote.
Disparlure	0	0	No potential risk can be identified.

[a] See Figure 3-1 for illustration.

[b] Hazard quotients less than 0.01 are given as zero. For *B.t.k.*, the lower range of the HQ is 0.000036. For NPV and disparlure, risks are essentially zero. For DDVP, exposure is unlikely and the risk is also essentially zero except for accidental exposures.

Table 4-1: Comparative hazard identification for potential effects in nontarget species

Endpoint	Agents used in Gypsy Moth Program						
	Gypsy Moth	*B.t.k.*	LdNPV	DFB	Tebufen-ozide	DDVP	Dispar-lure
Terrestrial species							
Mammals	■	○	○	●	●	●	○
Birds	■	○	○	□	■	●	○
Nontarget *Lepidoptera*	■	●	○	●	●	●	■*
Other arthropods	□	○	○	●	●	●	○
Other invertebrates	□	□	○	○	○	●	○
Plants	●	○	○	○	○	○	
Microorganisms	■	○		□			
Aquatic species							
Fish	□	○	○	□	□	●	○
Invertebrates	□	■	○	●	●	●	■
Plants	□	○	○	□	■		
Microorganisms	■	○		□			

* Effects in other pest *Lepidoptera* pest species only.

Key:	
●	Direct effects demonstrated in species of concern
■	Effects are plausible
□	Marginal evidence for effect
○	No plausible basis for risk
Blank	No data are available

Table 4-2: Comparative exposure assessment for ecological effects

Agent	Plausibility of Exposure	Primary Route	Comments
Gypsy moth	Variable	N/A	Exposure potential is high during outbreaks and decreases as intensity of infestation decreases.
B.t.k.	High	Oral	During broadcast applications, exposure potential is high.
LdNPV	High	Oral	During broadcast applications, exposure potential is high and can be reasonably well characterized.
Diflubenzuron	High	Oral	Can persist on vegetation and water contamination is plausible.
Tebufenozide	High	Oral	Can persist on vegetation and water contamination is plausible.
DDVP	Very low	Inhalation /Oral	Except in cases of insects entering the trap or other animals tampering with trap, exposures will be very low.
Disparlure	Variable	Variable	Very little compound is used in traps and exposures are likely to be very low.

Table 4-3: Comparative dose-response assessment for potential effects in nontarget species

Endpoint	Agents used in Gypsy Moth Program							
	Gypsy Moth	*B.t.k.*	Ld-NPV	DFB	4-CA	Tebufen-ozide	DDVP	Dispar-lure
Terrestrial species								
Mammals		■/□ [a]	□	■	■	■	■	
Birds		□		■	■	■		
Nontarget *Lepidoptera*	■	●		●		○		
Other arthropods				●		□		
Other invertebrates				□		○		
Plants	●							
Microorganisms				■				
Aquatic species								
Fish		□/○ [b]	□	○/■ [c]	□	■	■	■
Invertebrates		□	□	●	□	■	○	■
Plants				□	□	□		
Microorganisms				□	○			

[a] NOEC value only for oral exposure. NOEC and LOEC for inhalation.
[b] NOEC value only for tolerant species. LOEC only for sensitive species.
[c] Effect level only for acute exposures.

Descriptive Key:		
	●	Effect and no-effect levels clearly identified. Response or differences in sensitivities among species can be quantified.
	■	Effect and no-effect levels identified.
	□	Based on no-effect level only.
	○	Based on effect level only.
	Blank	No quantitative dose-response assessment is made.

Table 4-4: Comparative risk characterization for terrestrial species [a]

Agent	Hazard Quotient (HQ) [b]		Comments
	Lower	Upper	
Gypsy Moth	0.25	**400**	All HQs based on defoliation. Lower HQ based on low infestation (5 egg masses/acre) in intermediate stands. Upper HQ based on damage to sensitive stands in an outbreak (up to 83% defoliation). Effects secondary to defoliation will occur in some animal populations.
B.t.k.	0.36	**9.4**	All HQs based on lethality to terrestrial invertebrates using 10% as a benchmark. A maximum mortality of 3.6% for tolerant invertebrates and 94% for sensitive invertebrates
LdNPV	0	0	No toxicity to terrestrial species is likely. The upper range of the HQ is 0.001 and is based on the consumption of contaminated vegetation and an acute free-standing NOAEL in mammals.
Diflubenzuron	0.18	**32**	All HQs based on responses in terrestrial invertebrates. The lower range is based on tolerant species and the upper range on sensitive species.
4-Chloroaniline	0	0.02	The upper range based on the consumption of fish by a predatory bird after an accidental spill (acute scenario).
Tebufenozide	0	**4**	The upper range is based on the consumption of contaminated vegetation by a large mammal after 2 applications at the maximum application rate. While not quantified, effects on some nontarget *Lepidoptera* are possible.
DDVP	0	0	Typically, exposures will be minimal. Insects entering the traps are likely to be killed.
Disparlure	0	0	No potential hazard can be identified except possible mating disruption in other pest *Lepidoptera*.

[a] See Figure 4-1 for illustration. Note that the magnitude of the HQ among different agents is not a measure of relative risk or severity of effects. See text for discussion.
[b] Hazard quotients less than 0.01 are given as zero. For tebufenozide, the lower range of the HQ is 0.0002. For 4-chloroaniline the lower range of the HQ is 0.00002. For NPV and disparlure, lower range of the HQs are essentially zero. For DDVP, exposure is unlikely and the risk is also essentially zero except for accidental exposures.

Table 4-5: Comparative risk characterization for aquatic species [a]

Agent	Hazard Quotient (HQ) [b]		Comments
	Lower	Upper	
Gypsy Moth	0	0	No basis for asserting that adverse effects will be observed.
B.t.k.	0	0.5	All HQs based on aquatic invertebrates. Lower range is 0.007 for tolerant species. The upper range is based on sensitive species
LdNPV	0	0	No basis for asserting that adverse effects will be observed. The upper range is 0.00003 and is based on a free-standing NOEC.
Diflubenzuron	0	**5**	Upper range is based on acute effects in sensitive aquatic invertebrates (*Daphnia*) after peak exposures.
4-Chloroaniline	0	0.2	Upper range is based on acute exposures to aquatic invertebrates and aquatic plants.
Tebufenozide	0	0.4	Upper range is based on longer-term toxicity in sensitive aquatic invertebrates.
DDVP	0	0 **8**	No risks are plausible in normal use. The HQ for aquatic invertebrates could reach up to 8 in accidental exposures.
Disparlure	0	0.4	Upper range based on acute exposures to sensitive aquatic invertebrates (*Daphnia*).

[a] See Figure 4-1 for illustration. Note that the magnitude of the HQ among different agents is not a measure of relative risk or severity of effects. See text for discussion.
[b] Hazard quotients less than 0.01 are given as zero.

Figures

Figure 3-1: Risk comparison for potential human health effects
Figure 4-1: Risk comparison for potential effects in terrestrial species
Figure 4-2: Risk comparison for potential effects in aquatic species

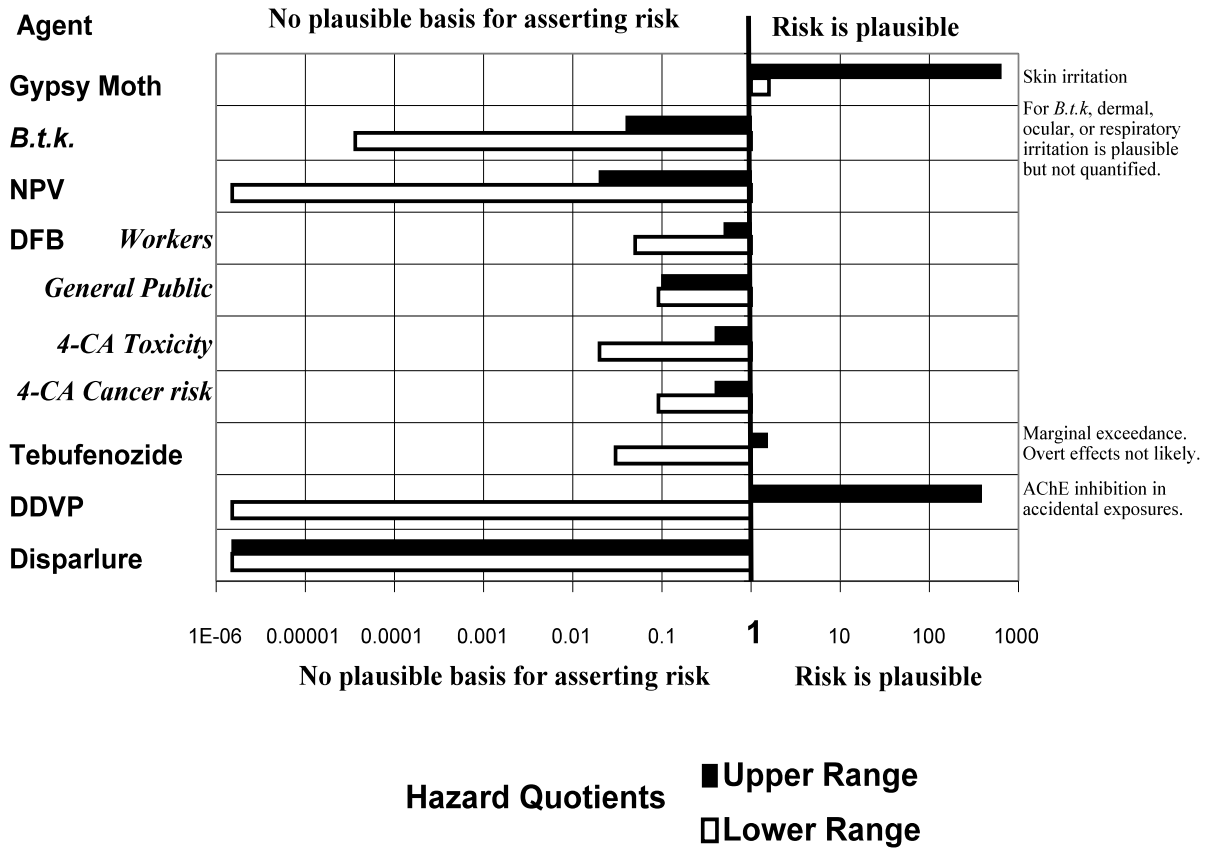

Figure 3-1: Risk comparison for potential human health effects.

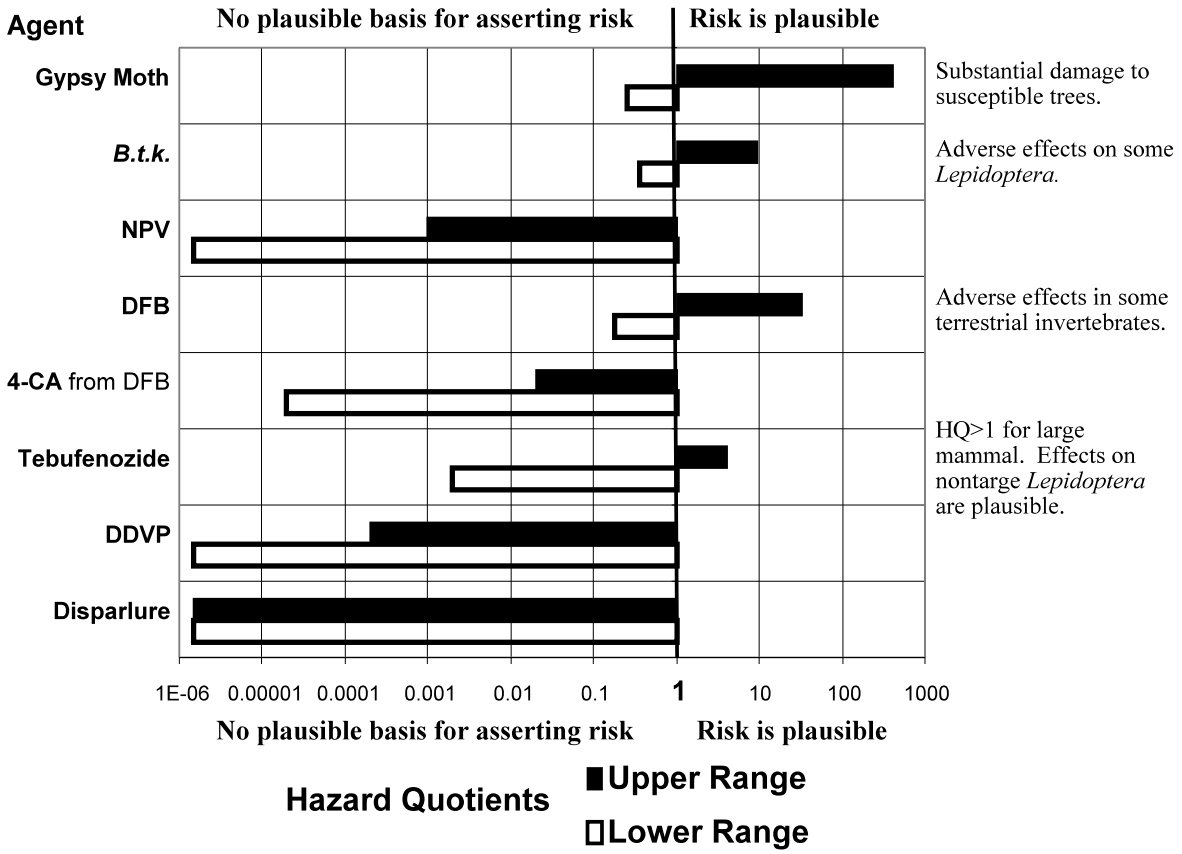

Figure 4-1: Risk comparison for potential effects in terrestrial species.

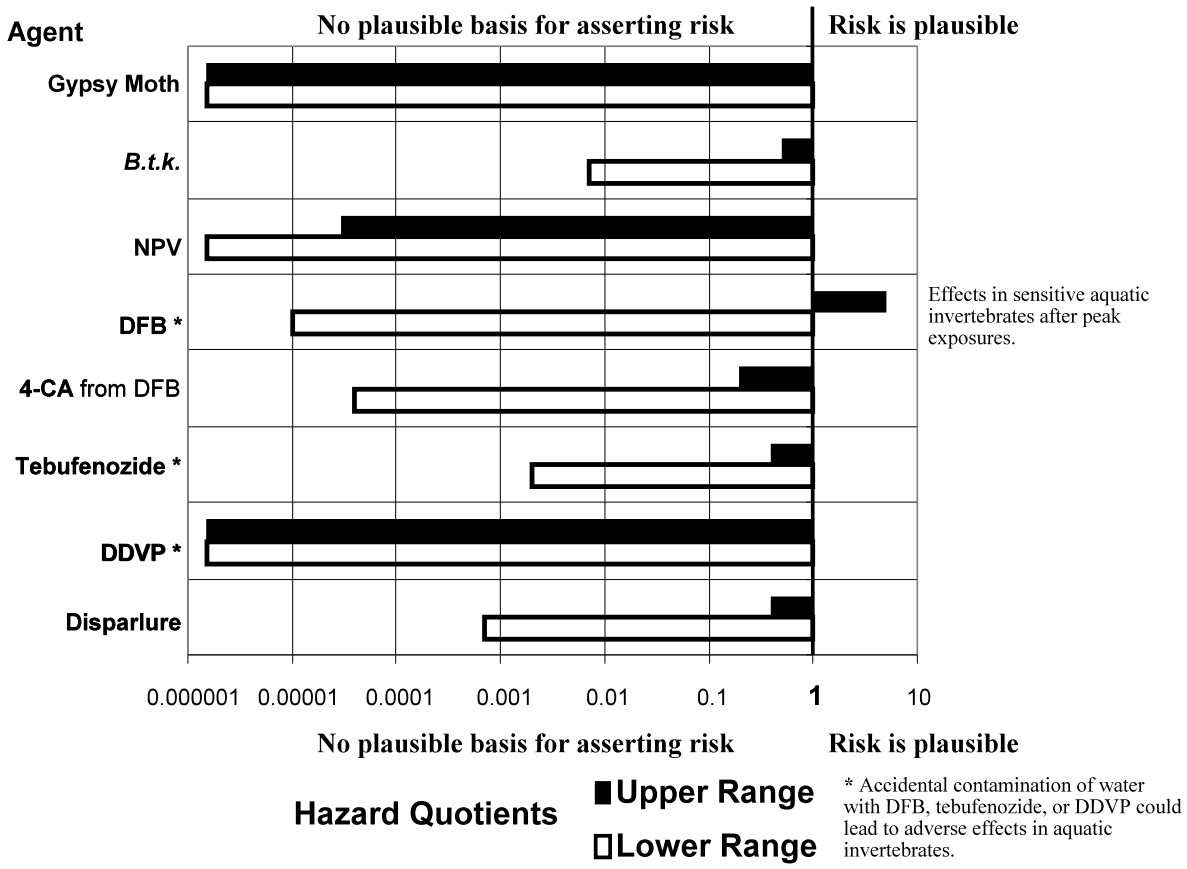

Figure 4-2: Risk comparison for potential effects in aquatic species.

Pesticide Precautionary Statement

Pesticides used improperly can be injurious to humans, animals, and plants. Follow the directions and heed all precautions on the labels.

Store pesticides in original containers under lock and key--out of the reach of children and animals--and away from food and feed.

Apply pesticides so that they do not endanger humans, livestock, crops, beneficial insects, fish, and wildlife. Do not apply pesticides when there is danger of drift, when honey bees or other pollinating insects are visiting plants, or in ways that may contaminate water or leave illegal residues.

Avoid prolonged inhalation of pesticide sprays or dusts; wear protective clothing and equipment if specified on the container.

If your hands become contaminated with a pesticide, do not eat or drink until you have washed. In case a pesticide is swallowed or gets in the eyes, follow the first-aid treatment given on the label, and get prompt medical attention. If a pesticide is spilled on your skin or clothing, remove clothing immediately and wash skin thoroughly.

Do not clean spray equipment or dump excess spray material near ponds, streams, or wells. Because it is difficult to remove all traces of herbicides from equipment, do not use the same equipment for insecticides or fungicides that you use for herbicides.

Dispose of empty pesticide containers promptly. Have them buried at a sanitary land-fill dump, or crush and bury them in a level, isolated place.

NOTE: Some States have restrictions on the use of certain pesticides. Check your State and local regulations. Also, because registrations of pesticides are under constant review by the Federal Environmental Protection Agency, consult your county agricultural agent or State extension specialist to be sure the intended use is still registered.

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