Gypsy Moth Management in the United States:
a cooperative approach

Final
Supplemental Environmental
Impact Statement

Volume III of IV

Appendixes F-I

Risk Assessments

United States
Department of Agriculture

Forest Service

Animal and Plant Health
Inspection Service

Newtown Square, PA

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Gypsy Moth Management in the United States:
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
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	Appendix K. DDVP (Dichlorvos) Risk Assessment
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	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 III

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

Final
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Bacillus thuringiensis kurstaki (B.t.k.) Risk Assessment

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Control/Eradication Agents for the Gypsy Moth -
Human Health and Ecological Risk Assessment for *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*)
FINAL REPORT

Prepared for:

USDA, Forest Service
Forest Health Protection

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GENERAL ACRONYMS, ABBREVIATIONS, AND SYMBOLS

a.i.	active ingredient
AEL	adverse-effect level
APHIS	Animal and Plant Health Inspection Service
ARS	Agricultural Research Station
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
B.t.k.	*Bacillus thuringiensis* var. *kurstaki*
BIU	Billions of international units
bw	body weight
cfu	colony forming units
cm	centimeter
DFB	diflubenzuron
EC_{50}	concentration causing 50% inhibition of a process
EC_{100}	concentration causing complete inhibition of a process
EEC	expected environmental concentration
EIS	environmental impact statement
F	female
F_1	first filial generation
FH	Forest Health
FS	Forest Service
FTU	forestry toxic units
g	gram
GC	gas chromatography
GRAS	generally recognized as safe
HQ	hazard quotient
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
i.p.	intraperitoneal
IU	international units
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
K_p	skin permeability coefficient
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
MW	molecular weight
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration

ACRONYMS, ABBREVIATIONS, AND SYMBOLS (*continued*)

NOEL	no-observed-effect level
NRC	National Research Council
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
OTS	Office of Toxic Substances
ppm	parts per million
RBC	red blood cells
RfD	reference dose
UF	uncertainty factor
U.S.	United States
U.S. EPA	U.S. Environmental Protection Agency
USDA	United States Department of Agriculture
$>$	greater than
$\geq$	greater than or equal to
$<$	less than
$\leq$	less than or equal to
$=$	equal to
$\approx$	approximately equal to

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

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

This document updates the human health and ecological risk assessments on *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*) prepared in 1995 in support of the Final Environmental Impact Statement (FEIS) for the Cooperative Gypsy Moth Management Program sponsored by the USDA Forest Service and APHIS. *B.t.k.* is used in USDA Forest Service and APHIS programs to control or eradicate the gypsy moth (*Lymantria dispar*). The updated risk assessments define the environmental consequences of using *B.t.k.* in these programs.

This is a technical support document and it addresses some specialized technical areas. Thus, parts of this document may contain information that is difficult for some readers to understand. These technical discussions are necessary to support the review of the document by individuals with specialized training. Nevertheless, an effort is made to ensure that the conclusions reached in the document and the bases for these conclusions can be understood by individuals who do not have specialized training in the chemical and biological sciences. In addition to this executive summary, each major section of the document starts with an overview section that is intended to summarize the technical discussion in a manner that most individuals will understand.

Sensitive terrestrial insects are the only organisms likely to be seriously affected by exposure to *B.t.k.* or its formulations. All sensitive terrestrial insects are lepidoptera and include some species of butterfly, like the endangered Karner blue and some swallowtail butterflies and promethea moths. 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. The risk characterization for other wildlife species is unambiguous: under foreseeable conditions of exposure, adverse effects are unlikely to be observed.

In terms of potential human health effects, formulations of *B.t.k.* are likely to cause irritation to the skin, eyes, and respiratory tract; however, serious adverse health effects are implausible. For members of the general public, exposure levels are estimated to be below the functional human NOAEL for serious adverse effects by factors of about 28,000 to 4,000,000 [4 million]. 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. This assessment is based on reasonably good monitoring data, conservative exposure assumptions, and an aggressive and protective use of the available toxicity data.

PROGRAM DESCRIPTION

Bacillus thuringiensis (*B.t.*) is a bacteria that is found in most of the world. Various strains of *B.t.*, including *B.t.k.*, are commonly found in soil, foliage, wildlife, water, and air. All commercial formulations of *B.t.k.* used by the USDA contain the HD-1 strain. Ten formulations of *B.t.k.* are used in USDA programs and all are supplied by Valent USA Corp or subsidiaries. Historically, each of the producers of *B.t.k.* formulations maintained separate stock strains and it appears that *B.t.k.* strain HD-1 may actually be a set of related strains or sub-strains.

B.t.k. formulations are complex chemical mixtures. *B.t.k.* is cultured or grown in a media containing water and nutrients including sugars, starches, proteins, and amino acids. These nutrients are themselves chemically complex and variable biological materials such as animal foodstuffs, a variety of flours, yeasts, and molasses. Relatively small quantities of essential elements, minerals, or salts also may be added to create optimal growth conditions. Other materials may also be used at various stages of production to enhance growth or facilitate the recovery of *B.t.k.* from the growth media. The other components of the formulation are mostly

water and a complex mixture of culture media and metabolites. The composition of the growth media used by a manufacturer may change over time, as different sources of nutrient material are used.

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. Typical application rates for *B.t.k.* range from 24 BIU/acre to more than 36 BIU/acre. The range of application rates used in the current risk assessment is 20 to 40 BIU/acre, which is equivalent to about 49 to 99 BIU/ha. Any preparation of bacteria carries the potential for contamination with other possibly pathogenic microorganisms, which must be addressed by proper quality control procedures. U.S. EPA requires that spore preparations of *B.t.* are produced by pure culture fermentation procedures with adequate quality control measures to detect either contamination with other microorganisms or changes from the characteristics of the parent *B.t.* strain. Although *B.t.k.* formulations may be applied by aerial spray or by ground spray, the number of aerial applications far exceeds the number of ground applications. More than 1 million pounds of *B.t.k.* are applied annually in the United States to control the gypsy moth. A total of 2,743,816 acres were treated with *B.t.k.* formulations between 1995 and 2002, for an average annual treatment rate of approximately 343,000 acres per year.

HUMAN HEALTH RISK ASSESSMENT

Hazard Identification – Most risk assessments for chemical and biological agents are based on relatively standard toxicity studies in experimental mammals. *B.t.k.*, however, is different in that several epidemiology studies – i.e., studies on populations of humans who have been exposed to *B.t.k.* – provide useful information regarding the plausibility of observing human health effects after *B.t.k.* applications that are identical or closely related to applications used in USDA programs to control the gypsy moth. The results of standard toxicity studies on *B.t.k.* and its formulations are used in this risk assessment to supplement information provided by epidemiology studies.

Irritation of the eyes, skin, and respiratory tract might be associated with exposures to *B.t.k.* and commercial formulations of *B.t.k.* Irritant effects are noted in experimental animal studies as well as in epidemiology studies and case reports. Other more serious signs of toxicity are not likely to occur as a result of human exposure to *B.t.k.* Specifically, there is little indication that *B.t.k.* is associated with pathogenicity in humans and no indication of endocrine disruption or reproductive effects in humans after exposure to *B.t.k.* formulations. In addition, carcinogenic and mutagenic effects are not likely to results from exposure to *B.t.k.* or its formulations. The potential for allergenicity of *B.t.k.* is somewhat more difficult to assess. There are reported incidents of potential skin sensitization and antibody induction in some individuals after exposure to *B.t.k.* formulations.

Exposure Assessment – Exposure assessments usually estimate the amount or concentration of an agent to which an individual or population might be exposed via ingestion, dermal contact, or inhalation. The exposure assessments are then compared with toxicity studies based on similar types of exposure—i.e., the dose-response assessment—and then the risk is quantified. The human health risk 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. Second, the apparent lack of a specific mechanism of toxicity for *B.t.k.* makes selecting the most appropriate measure of exposure somewhat arbitrary.

Dose-Response Assessment – Based on conclusions reached by the U.S. EPA and World Health Organization that irritation of the skin, eyes, or respiratory tract are most likely the only human health effects to be expected from exposure to *B.t.k.*, the dose-response assessment is relatively simple. Moreover, there is no information from epidemiology studies or studies in experimental mammals that *B.t.k.* is likely to cause severe adverse health effects in humans under any set of plausible exposure conditions. Notwithstanding these assertions, a recent epidemiology study suggests that the irritant effects of *B.t.k.* may occur with notable frequency at exposure levels that are typical of those used in programs to control the gypsy moth. By comparison, a study in workers demonstrates that the frequency of the irritant effects does not increase substantially even at very high exposure levels. This lack of a strong dose-response relationship is somewhat unusual but is consistent with experimental data in mammals.

Based on recent experimental studies which are not typically used in a quantitative dose-response assessment, it is possible to define very high exposure levels for *B.t.k.* which might pose a serious health hazard and it is possible to define a NOAEL for such effects that is consistent with the available human data. The exposure data are expressed in units of colony forming units (cfu). Specifically, cumulative exposures of up to 1.4×10^{10} cfu/m^3 × hour are not likely to result in adverse effects.

The same study that can be used to derive this NOAEL also suggests that pre-exposure to viral infections of the respiratory tract may increase the risk of serious adverse effects, including mortality in experimental mammals. While the dose-response relationship can be defined for a specific exposure scenario—i.e., exposure of mice to 4% of the LD_{50} of an influenza virus—these data are not directly or quantitatively applicable to the human health risk assessment.

Risk Characterization – 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. Nonetheless, more recent information alters the approach taken to quantifying the risk of exposure-related irritant effects and more serious health effects, thereby affecting the risk characterization. Unlike the previous USDA risk assessment, there is no attempt to quantify the risk of irritant effects. This approach is taken because the threshold for these effects cannot be determined. At application rates similar to those conducted by USDA in programs to control or eradicate the gypsy moth, some members of the general public as well as workers are likely to experience throat irritation, which is the best documented effect in the *B.t.k.* literature on human health effects. Nonetheless, dermal and ocular irritation are also likely effects, although perhaps only at the extreme upper levels of exposure.

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 of 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 adversely affected 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.

Pre-treatment with an influenza virus substantially increased morality in mice exposed to various doses of *B.t.k.* 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.

ECOLOGICAL RISK ASSESSMENT

Hazard Identification – 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 persistent in mammals for several weeks after exposure, there is little indication that oral or dermal exposure leads to any 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 the 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 non-target lepidoptera. Sensitive non-target 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 non-target 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 *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 be tested.

The U.S. EPA (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 process. 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.

Exposure Assessment – 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 of 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 to 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., about 49 to 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.

Dose-Response Assessment – The dose-response assessment parallels the exposure assessment. Specific dose-response assessments are presented for three groups: small mammals, terrestrial insects, and aquatic animals. For small mammals, dose-response assessments are given for inhalation and oral exposure. The risk assessment for inhalation exposure is based a mouse study in which mortality increased significantly after intranasal instillations of *B.t.k.* A dose of 10^7 cfu/mouse is taken as the NOAEL and 10^8 cfu/mouse is taken as a frank effect level —a dose associated with 80% mortality. The risk assessment for oral exposure, on the other hand, is based on a free-standing NOAEL, which is to say that there is no evidence that oral exposure levels, however high, will cause adverse effects in mammals or birds. For this risk assessment, the dose of 8400 mg/kg/day is used as the NOAEL. For terrestrial invertebrates, sufficient data are available to estimate dose-response relationships for sensitive species as well as for relatively tolerant species. Sensitive species, which consist entirely of lepidoptera, have an LD_{50} value of about 21 BIU/ha. Tolerant species, which consist of some lepidoptera and other kinds of terrestrial insects, have an LD_{50} of about 590 BIU/ha, which is about 28 times greater than the LD_{50} value for sensitive species. For both sensitive and tolerant species, dose-response curves are developed which permit mortality estimates for any application rate. As with terrestrial insects, dose-response assessments are provided for tolerant and sensitive species of fish and aquatic invertebrates. Fish appear to be somewhat less sensitive than invertebrates to *B.t.k.*. For tolerant species of fish, the NOEC is taken as 1000 mg/L, which corresponds to 2.5×10^{10} cfu/L, and is taken from a study in mosquito fish. For sensitive species of fish, the LOEC is based on a trout study in which marginally significant mortality was observed at 1.4 mg/L or about 2.87×10^7 cfu/L. The most sensitive invertebrate species appears to be *Daphnia magna*, with a chronic NOEC of 0.45 mg/L or 6.24×10^8 cfu/L for reproductive effects and mortality. The NOEC for tolerant species is taken as 36 mg/L based on bioassays in mayflies and caddisflies.

Risk Characterization – 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. 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—i.e., Collembola or earthworms—are plausible.

1. INTRODUCTION

This document updates the human health and ecological risk assessments on *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*) prepared in 1995 in support of the Final Environmental Impact Statement (FEIS) for the Cooperative Gypsy Moth Management Program (Durkin et al. 1994; USDA 1995) sponsored by the USDA Forest Service and APHIS. *B.t.k.* is used in USDA Forest Service and APHIS programs to control or eradicate the gypsy moth (*Lymantria dispar*). The updated risk assessments define the environmental consequences of using *B.t.k.* in these programs.

This is a technical support document and it addresses some specialized technical areas. Thus, parts of this document may contain information that is difficult for some readers to understand. These technical discussions are necessary to support the review of the document by individuals with specialized training. Nevertheless, an effort is made to ensure that the conclusions reached in the document and the bases for these conclusions can be understood by individuals who do not have specialized training in the chemical and biological sciences. Each major section of the document starts with an overview section that is intended to summarize the technical discussion in a manner that most individuals will understand. In addition, 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). Some of the more complicated terms and concepts are defined, as necessary, in the text.

In the preparation of this risk assessment, literature searches of *B.t.k* were conducted in the open literature using PubMed, TOXLINE, AGRICOLA, as well as the U.S. EPA CBI files. The body of literature regarding the environmental fate and toxicology of *B.t.k* is expansive.

In addition to the previously prepared risk assessments (Durkin 1994; USDA 1995), there are several books (Entwistle et al. 1993; Hickle and Fitch 1990; Glare and O'Callaghan 2000) and a relatively comprehensive review by the World Health Organization (WHO 1999) concerning the toxicology, environmental fate, and other issues associated with the use of *B.t.*, including *B.t.k.* Several other reviews of various topics involving *B.t.* are published in the open literature (e.g., Addison 1995; Auckland District Health Board 2002; Drobniewski 1994; McClintock et al. 1995b; Meadows 1993; Siegel 2001; Swadener 1994).

Also, numerous studies were submitted to the U.S. EPA/OPP in support of the reregistration of *B.t.,* and most of these studies are reviewed in U.S. EPA (1998), which summarizes the product chemistry, mammalian toxicology, and ecotoxicology studies submitted by industry. The U.S. EPA Office of Pesticide Programs kindly provided the full text copies of most of these studies (n=222). The CBI studies were reviewed during the preparation of this risk assessment, and synopses of the information that can be disclosed from these studies are included in this document.

Genetic material from *B.t.k.* is incorporated into some food crops. In its evaluation of the process, the U.S. EPA concluded that although the endotoxin is not toxic to mammals or other vertebrates, it may be toxic to lepidopteran species (U.S. EPA 2000a) . For the most part, this risk assessment does not address the use of *B.t.k.* toxins in food crops (e.g., Raps et al. 2001; Wraight et al. 2000); however, certain studies involving transgenic food crops (Fares and El-Sayed 1998; Yu et al. 1997) are considered because they are relevant to the hazard identification for humans and non-target mammalian species.

While this document discusses the studies used to support the risk assessments, it makes no attempt to summarize all of the information cited in the existing reviews. This is a general

approach in all Forest Service risk assessments. For *B.t.k.* in particular, an attempt to summarize all of the available data would tend to obscure the key studies which should and do have an impact on the risk assessment.

The Forest Service updates their risk assessments periodically and welcomes input from the general public regarding the selection of studies included in the risk assessment. This input is helpful, however, only if recommendations for including additional studies specify why the new or not previously included information is likely to alter the conclusions reached in the risk assessments.

The risk assessment methods used in this document are similar to those used in risk assessments previously conducted for the Forest Service as well as risk assessments conducted by other government agencies. Details regarding the specific methods used to prepare the human health risk assessment are provided in SERA (2001). 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 *B.t.k*. and its commercial formulations, 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.

Variability can be a dominant factor in any risk assessment. The current risk assessment addresses variability as appropriate. Within the context of this risk assessment, variability has a minimal impact on the human health risk assessment. As discussed in Section 3, the human experience with *B.t.k.* applications allows for a relatively unambiguous assessment of risk. In the ecological risk assessment (Section 4), the major source of variability involves differences among and within groups of organisms. For terrestrial insects which comprise the basic group most likely to be affected directly by *B.t.k.* applications, data are adequate to derive separate dose-response curves for sensitive and tolerant species and to suggest possible distributions of tolerance for species with intermediate sensitivity. For other groups, the data are less detailed but some attempt is made to express differences within groups when appropriate.

2. PROGRAM DESCRIPTION

2.1. Overview

Bacillus thuringiensis (*B.t.*) are naturally occurring bacteria that can be found in soil, foliage, wildlife, water, and air. All commercial formulations of *B.t.k.* used by the USDA contain the HD-1 strain. Historically, each of the producers of *B.t.k.* formulations maintained separate stock strains. Based on an analysis of cellular fatty acids in various commercial and standard cultures of *B.t.k.*, it appears that *B.t.k.* strain HD-1 may actually be a set of related strains or sub-strains. Ten different formulations of *B.t.k.* are used in USDA programs and all are supplied by Valent USA Corp or subsidiaries. Typical application rates for *B.t.k.* range from 24 BIU/acre to more than 36 BIU/acre. The range of application rates used in this risk assessment is 20 to 40 BIU/acre, which corresponds to approximately 49 to 99 BIU/ha. Since any preparation of bacteria has the potential for contamination with other possibly pathogenic microorganisms, U.S. EPA requires that spore preparations of *B.t.* are produced by pure culture fermentation procedures with adequate quality control measures to detect either contamination with other microorganisms or changes from the characteristics of the parent *B.t.* strain. Although *B.t.k.* formulations may be applied by aerial spray or by ground spray, the number of aerial applications far exceeds the number of ground applications. More than 1 million pounds of *B.t.k.* are applied annually in the United States to control the gypsy moth. A total of 2,743,816 acres were treated with *B.t.k.* formulations between 1995 and 2002, for an average annual treatment rate of about 343,000 acres per year.

2.2. Chemical Description and Commercial Formulations

Bacillus thuringiensis (*B.t.*) are rod-shaped, gram-positive, spore-forming aerobic bacteria found in most of the world (Cheon et al. 1997). *B.t.* was first isolated from diseased silk worms in Japan in 1901. In 1915, Berliner isolated *B.t.* from diseased flour moths. Depending on the classification systems used, between 1600 and 40,000 strains of *B.t.* have been isolated (Addison 1995). The vegetative cells are 1 μm wide, 5 μm long, and have flagellae, which are short hair-like structures used for locomotion. Various strains of *B.t.*, including *B.t.k.* , are ubiquitous in the environment and can be isolated from soil, foliage, wildlife, water, and air (Damgaard et al. 1997b; Iriarte et al. 1998; Maeda et al. 2000; Martin 1994; Swiecicka et al. 2002).

B.t.k. was first isolated in France by Kurstak in 1962. A new strain of *B.t.k.* was identified in the pink bollworm and named the HD-1 strain by Dulmage et al. (1971). All commercial formulations of *B.t.k.* used by the USDA contain the HD-1 strain (U.S. Department of Agriculture, Forest Service 1994a). The HD-1 strain produces the Cry1Ac, Cyr1Aa, Cry2Aa, and Cyr2Ab delta-endotoxins (Saxena et al. 2002) as well as chitinase (Wiwat et al. 2000). Different serotypes of *B.t.k.* , in addition to HD-1, have been identified (Lee et al. 2001; Li et al. 2002).

Some strains of *B.t.* contain the beta-exotoxin, which is mutagenic in mammals (Meretoja et al. 1977). Such strains are not permitted commercial formulations of *B.t.k.* that are sold in Canada or the United States (British Columbia Ministry of Health 1992, U.S. EPA 1988b). Batches of commercial *B.t.k.* are assayed for beta-toxins to ensure that the commercial batches do not contain the beta-exotoxin (Chen et al. 1990k; Chen et al. 1990l; Isaacson 1991b).

Historically, each of the producers of *B.t.k.* formulations maintained separate stock strains (e.g., Smith and Regan 1990k; Smith and Regan 1990m; Smith and Regan 1990n). The U.S. EPA (1998, pp. 3-4) RED on *B.t.* designates eight different strains of *B.t.k.* The identity of commercial strains is based on flagella antigen serotyping (Chen and Macuga 1990o; Chen and Macuga 1990p; Chen and Macuga 1990q), endotoxin characteristics (Chen and Macuga 1990r;

Chen and Macuga 1990s; Chen and Macuga 1990t; Fitch et al. 1990; Swysen and Hoogkamer 1991) and differential sensitivity to antibiotics (Smith and Regan 1989d; Smith and Regan 1989e; Smith and Regan 1989f).

Analysis of cellular fatty acids in various commercial and standard cultures of *B.t.k.*, suggests that *B.t.k.* strain HD-1 may actually be a set of related strains or sub-strains (Siegel et al. 2000). The U.S. EPA (1998) discontinued the grouping of isolates under subspecies names because the genetic material for delta endotoxins resides in plasmids that can be transferred from one isolate to another.

As discussed in Section 4, there is concern that heat stable toxins may occur in some batches of *B.t.k.* Most *B.t.k.* toxins are heat labile—i.e., the insecticidal/toxic activity of the toxins are destroyed by autoclaving (e.g., Chen et al. 1990h; Chen et al. 1990i; Chen et al. 1990j).

Table 2-1 provides a list of the specific *B.t.k.* formulations registered for control of the gypsy moth in forestry applications. Typically, the potency of commercial formulations of *B.t.k.* is expressed as BIU/gallon of formulated product or BIU/pound of formulated product. The term *BIU* is an acronym for billions of international units. This potency is measured in a bioassay using the cabbage looper (Dulmage et al. 1971). During production and formulation, each commercial batch of *B.t.k.* is used in the bioassay to determine the LC_{50} for the test insect, expressed as mg product/kg diet. The potency of the batch is then adjusted to the nominal requirement, as specified for the various formulations listed in Table 2-1. Hence, the use of BIU/acre to express an application rate is meaningful in terms of insecticidal efficacy, assuming that toxic potency to the gypsy moth is related to the toxic potency of *B.t.k.* to the test species used in the bioassay of the formulation. The potency of *B.t.k.* formulations varies from about 14 to about 48 BIU/lb formulated product. The label for Foray 48F specifies potency in units of Forestry Toxic Equivalents [FTUs]. FTU is a measure of potency similar to BIU except that the bioassay is based on the gypsy moth rather than the cabbage looper. This approach is taken because some formulations such as Foray 48F contain different ratios of crystals that are more effective against forestry pests (i.e., the gypsy moth and tussock moth) rather than agricultural pests (e.g., the cabbage looper). Typical application rates for *B.t.k.* expressed in units of BIU range from 24 to more than 36 BIU/acre (USDA Forest Service. 1999). The range of application rates used in this risk assessment is 20 to 40 BIU/acre, which is equivalent to about 49 to 99 BIU/ha [i.e., 2.471 acres per hectare].

As indicated in Table 2-1, the commercial formulations of *B.t.k.* contain between 3.5% and 10.3% protein toxins—i.e., the delta-endotoxin. The remainder of the formulations consists of materials that are classified as *inerts*. The inerts in *B.t.k.* formulations are discussed in Section 3.1.15 of this risk assessment.

The chemical and biological variability of *B.t.k.* formulations is not well characterized. One index of variability, however, is the number of viable spores in the formulation. Because the viable spores, together with the crystalline toxins, are agents that exert a toxic effect on the gypsy moth, there are some data regarding the number of spores in various formulations. For Foray 48B, microbial analyses of individual batches over a 2-year period indicate that the number of spores per unit of weight of the formulation can vary by a factor of 50 (Overholt 1994).

Any preparation of bacteria has a potential for contamination with other possibly pathogenic microorganisms, and this concern must be addressed by proper quality control procedures (Bernhard and Utz 1993). Between 1985 and 1987, random samples of *B.t.k.* purchased by the various states or provinces were found to contain various bacterial contaminants, although none

were considered pathogenic. In response to the concerns raised by this contamination, manufacturers took steps in 1988 to ensure that each batch of *B.t.k.* is free of detectable levels of contaminants. Since 1988, no substantial levels of bacterial or yeast contaminants were found in *B.t.k.* samples (Reardon et al. 1994). As part of an epidemiology study conducted by Noble et al. (1992), Foray 48B samples were tested and found to contain no other bacteria.

U.S. EPA (1988b) requires that spore preparations of *B.t.* are produced by pure culture fermentation procedures with adequate quality control measures to detect either contamination with other microorganisms or changes from the characteristics of the parent *B.t.* strain. In addition, prior to final formulation, each lot must be tested by subcutaneous injection of at least 1 million spores into at least five mice.

2.3. Use Statistics

Although *B.t.k.* formulations may be applied by aerial spray or by ground spray, the number of aerial applications far exceeds the number of ground applications. More than 1 million pounds of *B.t.k.* are applied annually in the United States to control the gypsy moth (Green et al. 1990). As indicated in Table 2-2, a total of 2,743,816 acres were treated with *B.t.k.* formulations between 1995 and 2002, for an average annual treatment rate of about 343,000 acres per year.

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 (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 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.

3. Human Health Risk Assessment

3.1. HAZARD IDENTIFICATION

3.1.1. Overview

Most risk assessments for chemical and biological agents are based on relatively standard toxicity studies in experimental mammals. *B.t.k.*, however, is different in that several epidemiology studies provide useful information regarding the plausibility of observing human health effects after *B.t.k.* applications that are identical or closely related to applications used in USDA programs to control the gypsy moth. The results of standard toxicity studies on *B.t.k.* and its formulations are used to supplement information provided by epidemiology studies.

In humans, irritation of the eyes, skin, and respiratory tract are effects that might be associated with exposure to *B.t.k.* and its commercial formulations. These irritant effects are reported in experimental animal studies as well as in epidemiology studies and case reports. The plausibility of such effects resulting from the use of *B.t.k* in USDA programs is considered further in the risk characterization (Section 3.4). Other more serious signs of toxicity are not likely to occur as a result of human exposure to *B.t.k.* Specifically, there is little indication that *B.t.k.* will be associated with pathogenic effects in humans and essentially no indication of endocrine disruption or reproductive effects in humans after exposure to *B.t.k.* Carcinogenic and mutagenic effects are not likely to be associated with exposure to *B.t.k.* or *B.t.k.* formulations. The potential for allergenicity is somewhat more difficult to assess in light of the reported incidents of potential skin and systemic sensitization and antibody induction in some individuals after exposure to *B.t.k.* formulations.

3.1.2. Epidemiology Studies

Epidemiology studies involve observations on human populations to assess whether or not a particular agent or exposure is associated with one or more effects. Case studies are different from epidemiology studies in that they generally involve reports of adverse effects in one or more individuals associated with a specific incident. Although case reports are discussed in the various subsections below, this section is restricted to the available epidemiology studies for which an overview is presented in Table 3-1. Most of the studies discussed compare the responses of populations exposed to aerial applications of *B.t.k.* formulations with responses of populations in unsprayed areas (e.g., Elliott et al. 1988; Noble et al. 1992; Aer'aqua Medicine Ltd. 2001). In one study, responses in a population are compared before and after application of a *B.t.k.* formulation (Petrie et al. 2003). A recent study in British Columbia (Pearce et al. 2002; Valadares de Amorim et al. 2001) concerns individuals in treated and untreated areas but focuses specifically on children with a history of asthma. Two studies involve workers, either individuals applying a *B.t.k.* formulation (Cook 1994; Noble et al. 1992) or workers harvesting crops that were treated with *B.t.k.* (Bernstein et al. 1999). This section focuses on a description of the individual studies. In the following subsections, this information is used in conjunction with the case studies and toxicology data in mammals to document the assessment of plausible effects.

The first substantial epidemiology study of *B.t.k.* applications was conducted in Oregon as part of a program to control a gypsy moth infestation (Elliott 1986; Elliott et al. 1988; Green et al. 1990). In the Oregon program, spray operations were conducted in April, May, and June of 1985 and 1986. *B.t.k.* was applied to more than 250,000 acres in 1985 and 270,000 acres in 1986. The *B.t.k.* was sprayed from helicopters in three separate applications (approximately 7 to10 days apart) over forest, rural, and urban areas. All spraying was conducted between daybreak and approximately 10:00 a.m. (Elliott et al. 1988). None of the publications on the Oregon Program reports the nominal application rate. According to the Oregon Department of

Agriculture, the application rate was 16 BIU/acre of a Dipel formulation. The health surveillance activities that accompanied the Oregon spray program are reported by Green et al. (1990). The total population of Lane County at the time of the study was 260,000. The 1985 spray covered an area with a population of approximately 80,000; the 1986 spray covered an area with a population of approximately 40,000. A surveillance program was established involving the four largest clinical laboratories in the area, three of which were associated with hospitals and one of which was an outpatient facility. All clinical cultures that were positive for any *Bacillus* species were subcultured, and the presence of *B.t.k.* in the subcultures was determined. As a control, the same procedure was followed for an unsprayed community approximately 60 miles from the spray area. No *B.t.k.* positive samples (n=7) were identified from the unsprayed community. In the samples from Lane County, a total of 55 *B.t.k.* positive cultures were found over the 2-year study period, 52 of which were associated with incidental contamination. Two of the three remaining samples may have been the result of contamination. The third sample was from an abscess in an IV drug user and "*..., B.t. could have been responsible for this localized infection, but it could also have been a skin or wound contaminant, or it could have colonized an abscess caused by another organism*." (Green et al. 1990, p. 851).

Another relatively large epidemiology study involving applications of *B.t.k.* formulations to control gypsy moth populations was conducted somewhat later in British Columbia (Bell 1994; Cook 1994; Noble et al. 1992). The aerial applications were conducted over a period of approximately 10 weeks, April 18 to June 30, 1992, at a rate of 50 BIU/ha or 20.2 BIU/acre (50 BIU/hectare ÷ 2.471 acres/hectare). According to records kept by a selected group of family practice physicians, there were no detectable effects of exposure among members of the general public (Noble et al. 1992). The records of 1140 physicians' office visits were reviewed. Of these, 675 were classified as clearly unrelated to symptoms that might be associated with the spraying. The remaining records involved reports of allergies, asthma, rhinitis, conjunctivitis, infections of the ear, sinus, or respiratory tract, and skin rashes. Although the available data did not permit an assessment of each individual's exposure to *B.t.k.*, available information on postal zones for each individual's residence suggested that the numbers of these complaints were evenly divided between individuals living inside and outside of the spray area. In addition, 3500 records of admissions to hospital emergency departments were reviewed. In no case was *B.t.k.* implicated as an agent causing any disease or clinical complaint.

An analysis of all *Bacillus* isolates from all the hospitals and laboratories in the study area indicated that many people were exposed to *B.t.k.*; however, in all cases, chromatography of cellular fatty acids indicated that the *B.t.k.* recovered from these sources was different from that used in the aerial spray (Noble 1994). Of 10 different vegetable samples assayed for *B.t.k.*, five were positive during the spray period. As with the *B.t.k.* recovered from human samples, the *B.t.k.* in the vegetable samples was different from the *B.t.k.* used in the aerial spray. This indicates that oral exposure to *B.t.k.* was common in this area but that this exposure was not attributable to the aerial spraying. As discussed in the program description (see Section 2), *B.t.k.* is commonly found in nature, and widespread incidental exposure to *B.t.k.* is to be expected. In no case was *B.t.k.* the agent causing an infection (Noble et al. 1992). When *B.t.k.* was recovered in stool samples, the medical histories did not suggest that the *B.t.k.* was associated with signs or symptoms of food poisoning or a disease with watery diarrhea similar to or suggestive of *Bacillus cereus.*

Some ground workers from the British Columbia study involved in the application of *B.t.k.* remained culture positive for long periods of time. Of 115 workers exposed to *B.t.k.* and available for follow-up studies, 15 yielded positive *B.t.k.* cultures from nose swabs 30 to 60 days after exposure. Five were positive at 120 days after exposure. No positive cultures were

identified after 140 days from the termination of exposure. Signs of respiratory or nasal infections and other health effects attributed to *B.t.k.* were not observed in any of the workers at any time (Cook 1994).

Similar results are reported by Bernstien et al. (1999) who studied various groups of workers involved in harvesting crops treated with Javelin, an agricultural formulation of *B.t.k.* that is not used in USDA programs. In this study, various crops (i.e., celery, parsley, cabbage, kale, spinach, and strawberries) were treated with the *B.t.k.* formulation at an unspecified application rate. The product label for Javlin (www.greenbook.net), indicates that the formulation is typically applied at a rate of about 0.12 to 1.5 lbs/acre. Since Javelin contains 17 BIU/lb, the likely rate used in these studies ranges from 2 to 25.5 BIU/acre.

The Berstien et al. (1999) study consisted of a longitudinal, follow-up investigation of 48 (46M, 2F) workers who were involved in picking *Bt*-sprayed crops (celery, parsley, cabbage, kale, spinach, strawberries) and who were tested during 4 visits: Visit 1(N=48, baseline 1, classified as Low for exposure), visit 2 (N=32, baseline 2, just prior to *Bt*-spraying, classified as Low for exposure), visit 3 (N=32, one month after *Bt*-spraying, classified as High for exposure) and visit 4 (N=20, 4 months after *Bt*-spraying, classified as High for exposure). Two additional groups were included: Group 2, Low (N=44) who handled a crop (onions) not *Bt*-sprayed and located 3 miles away from Bt-sprayed fields; and a Group 3 Medium (N=34), who washed and packed *Bt*-sprayed vegetables. Tests included a clinical evaluation for the presence of allergy or atopy, skin-prick tests to *B.t.k.* and non-*B.t.k.* (control) extracts, blood testing for IgE and IgG antibodies specific to a) Javelin water-soluble pesticide extracts (J-WS); b) Javelin-mercaptoethanol-sodium dodecyl sulfate (J-ME-SDS); Javelin proteinase K spore extracts (J-PK); and Javelin-associated pro-delta-endotoxin (J-PROTOX), and nasal and mouth lavages for bacterial counts. As is the case with the study by Cook (1994), nasal cultures were positive for *B.t.k.* in 66% of the high exposure workers 1 month after exposure. Positive *B.t.k.* nasal cultures were also noted in other groups and a statistically significant ($p<0.05$) association was noted with respect to the qualitative exposure groups. While the atopic status was similar across all groups of workers, Bernstien et al. (1999) classify 3 of 9 workers who handled *B.t.k.*-treated vegetables (parsley, spinach or celery) reporting clinically defined skin manifestations due to irritant/contact dermatitis of the forearms after contact at work with the vegetables. It is not clear, however, whether these were incidences of contact dermatitis due to *B.t.k.* exposure or whether they reflect skin contact sensitivities to the vegetables alone. Thirteen of the 32 Group 1workers (~40%) who were tested on two occasions (baseline and 1 month after spraying) converted from skin-prick negative (baseline) to skin-prick positive while 3 of 4 workers who were positive at baseline remained positive. Similarly, of the 20 workers who were serially (longitudinal study) tested on all three visits (baseline, and at 1 and 4 months after spraying), 13 (65%) converted from negative to positive reactions, whereas skin test conversions from positive to negative occurred in two workers. Thus, the number of positive skin-prick tests to both J-WS and J-ME-SDS extracts but not to J-PK and J-PROTOX increased 1 month after exposure and persisted for 4 months after exposure to Javelin spray. Taken together these studies indicate that while a small number of workers were sensitized to *B.t.k.* prior exposure, *de novo* sensitization occurred in a significant number of workers following exposure to an aerial spray of *B.t.k.* formulations.

Data on the development of IgE and IgG antibodies specific to various *B.t.k.*-related antigens are less clear since these data suffer from a significant non-random loss of sera which were not available for testing at various points of the study. This is especially true for Group 1, visit 3 at 4 months after spraying in which the number of sera tested dropped from 22 to 8 for IgE and to 6 for IgG. Therefore, the results presented in Bernstien et al. (1999, Table 5, page 579) should be interpreted with caution. It is evident that in the longitudinal study of Group 1, the number

of IgE-positive sera to J-WS increased significantly after exposure compared to baseline values ($p<0.05$). The cross-sectional study in which Group 1is compared to Groups 2 and 3, indicated that the incidence of IgE-positive sera in Group 1 was significantly higher from that in Groups 2 and 3 for both the J-WS and J-ME-SDS antigens while results with BtkVeg and BtaVeg antigens were not significantly different among the 3 Groups. Of significance to this review is the observation that the sera of 10 workers tested at pre-exposure and at 4 months after exposure showed a significant increase in IgE-specific titres (prior exposure OD, 0.08 ±0.01 SEM; post-exposure: mean OD, 0.22 ±0.07 SEM, compared to 14 non-exposed urban controls; mean OD 0.12 ±0.01 SEM). This clearly reflects an anamnestic response – i.e., a late response to antigen. In contrast, data on the IgG response indicated that the incidence of IgG-positive sera from Group 1 workers was high at baseline and remained high in all subsequent visits. In the cross-sectional study of all exposure groups the incidence of IgG-positive titres specific for J-WS was significantly higher compared to Group 2 (control) whereas the incidence of IgG-positive titres specific for J-ME-SDS was significantly higher compared to Groups 2 and 3. These data suggest that workers in Group 1 may have been exposed previously to *B.t.k.* which resulted in a substantial number of these producing IgG antibodies to a variety of *B.t.k* components and that a further increase in antigen-specific IgG antibodies upon re-exposure was minimal. Thus, it is clear from this study that exposure to *B.t.k.* may result in sensitization of workers as indicated by the increase in IgE titres following exposure. It is less clear, however, whether the presence of IgE antibodies would result in clinical manifestations of allergy. From the data presented in the Bernstein et al. (1999) study it is evident that an increase in IgE titers from 0.08 to 0.22 occurred in pre- to post-exposure workers without any clinically defined exposure-associated manifestations of allergy. The possibility exists that levels of IgE antibodies may increase upon repeated exposures.

However, as has been observed in the Laferriere et al. (1987) study, antibody titres are reduced rapidly after exposure has ceased and the probability that this would result in clinically defined allergenicity in these workers would be low. This study included workers who took part in the Quebec Ministry of Energy and Resources (M.E.R.) spraying program which lasted for two years (May 1994 – June 1995). Sera from 112 workers (manual/technical laborers) were tested for antibody to *B.t.k.* vegetative cells or to spores or to a spore-crystals mixture. This study's results should be interpreted with caution since several sera are missing throughout the testing period, and the class of *B.t.k*-antibodies – i.e. reaginic (IgE) or IgG – is not reported. A small number (5/112 or 5%) of workers who were tested in May 1994 (start of the spraying) and in June 1994 (middle of the activity) were reported to be positive for antibodies to vegetative cells by June 1994. Of the 5 positive subjects, the titre in worker #12 in June was the same as that in May, in workers #23 and #29 doubled in June over that in May, and in workers #16 and 24 titers in June were 1/80 and 1/160 respectively but for these workers titres were not available for May. Weak titres of 1/20 to spores and spores-crystals mixture were recorded only in worker #29 by June but sera were not analyzed in May for this subject. Three of these workers (#12, 16 and 23) were followed up during the next year's activity (sera were collected in May, July and September 1995). Workers # 12 and 23 showed an increase in titres to vegetative cells by July, while the titre to vegetative cells in worker #16 was higher in May compared to July. The titres in all three workers decreased by September. Worker #16 who was negative in June 1984 to spores-crystals antigens became weakly positive to the same antigens by July 1985 and remained positive in September 1985. Worker #19, who was not tested in 1984, had a titre of 1/320 by May 1985 and was reduced by September 1985. Serum for July 1985 was not available. Five additional workers (technicians) who were tested in 1985 were negative for antibodies to vegetative cells and spores. These, however, were weakly positive (titre of 1/20) in May to the spores-crystals mixture. In June 1986 (approximately 1 year after exposure), sera from three manual laborers who had strongly reacted in the 1985, were re-tested and found to

be negative for all three antigens. This study did not report any exposure-related clinical manifestations in these workers. Collectively, these data suggest that a small number of workers become sensitized to *B.t.k.* constituents and that upon re-exposure the antibody levels increase transiently, decrease within a month, and are undetectable after one year.

An epidemiology study specifically designed to assess potential effects of *B.t.k.* exposure on children with asthma was conducted in Vancouver Island, British Columbia (Pearce et al. 2002). In this study, 29 children with asthma were identified in the area to be treated and were matched to 29 children with asthma outside of the spray area. Endpoints examined included recorded symptoms and peak expiratory flow rates. The spray zone and no spray zone were separated by 1 kilometer. Exposures were assessed by Kromecote cards, air concentrations of *B.t.*, and nasal swabs. The treated area received three sprays of Foray 48B at a rate of 4 L/ha. This is equivalent to approximately 8.452 pints per 2.471 acres or 3.4 pints/acre, in the mid-range of the application rate used in Forest Service programs—i.e., 1.3 to 6.7 pints/acre (Table 2-1). Three separate applications were made at 10-day intervals. There were no apparent differences between the children in treated and untreated areas with regard to asthma symptoms or peak respiratory flow rates. It is noteworthy that children in the "non-treated" areas did receive some level of exposure to *B.t.k.* based on Kromecote cards (78% positive in treated area and 9% positive in untreated area) as well as positive cultures from nasal swabs. It is also interesting that five nasal swabs were positive for *B.t.k.* prior to any spray. The average concentration of *B.t.k.* in the spray zone was 739 cfu/m^3 during spraying. Monitoring data regarding *B.t.k.* concentrations in air are reported also by Teschke et al. (2001). Although it appears that both groups of children were exposed to B.t.k., there was an apparent lack of increased symptoms in either group. Consequently, the study by Pearce et al. (2002) seems to demonstrate that adverse effects were not associated with the *B.t.k.* spray.

Another large epidemiology study conducted in New Zealand (Aer'aqua Medicine Ltd. 2001). This study involves a program in which Foray 48B was sprayed for the control of the white-spotted tussock moth in two regions of New Zealand during 1996 and 1997. The total exposed population was comprised of approximately 88,000 individuals. During the spray program, self-reports of adverse reactions were recorded and sentinel physicians were actively used to assess changes in disease pattern. After the spray program, records of reported diseases were reviewed and the incidence of birth outcomes were analyzed. No effects were noted based on reported cases of anaphylaxis from sentinel physicians, incidences of birth defects or changes in birth weight, the incidence of meningococcal disease, or reported infections with *B.t.k.* Among 375 self-reported incidents of potential adverse effects, the only notable response was an increase in respiratory, dermal, and ocular irritation. All applications appear to have been made at the rate of 5 L/ha of Foray 48B (Aer'aqua Medicine Ltd. 2001, Appendix 6, Appendices p. 10), which is equivalent to about 10.6 pints (2.113 pints/L) per 2.471 acres or 4.3 pints Foray 48B per acre. As indicated in Table 2-1, this application rate is within the upper range of application rates typically used to control gypsy moth infestations—i.e., 1.3 to 6.7 pints/acre.

Petrie et al. (2003) conducted another epidemiology in New Zealand, which is somewhat smaller than the study by Aer'aqua Medicine Ltd. (2001) and involves only self-reporting surveys of symptoms. A major difference in the Petrie et al. (2003) study, however, is that the investigators surveyed the same individuals both before (n=292) and after (n=181) the application of Foray 48B. Several of the 25 endpoints surveyed by Petrie et al. (2003) are classified as statistically significant—i.e., sleep problems, stomach discomfort, irritated throat, itchy nose, dizziness, diarrhoea, "gas discomfort", extra heart beats, and difficulty concentrating. The investigators categorize these effects into three general classes: irritant effects, gastrointestinal effects, and effects characterized as neuropsychiatric—i.e., sleep

disorder, difficulty in concentrating, and dizziness. A significant increase was noted in participants with a history of hay fever (p=0.02) after spraying compared with those participants not previously diagnosed with hay fever. There was no significant increase in the number of participants with a history of asthma (p=0.14) or other allergies (p=0.22) when compared with participants without these diagnoses (Petrie et al. 2003, page 4). The increase in hay fever could be incidental, since the pollen season in Aukland is from October to February and this may have influenced upper airway and hay fever symptoms reported by the participating workers.

Petrie et al. (2003) recommend caution when interpreting this kind of self-reporting survey because only about 62% of the individuals in the pre-application survey responded to the post-application survey, and, in self-reporting studies such as this, individuals who feel they were adversely affected by exposure are more likely to respond in the post-application survey. Petrie et al. (2003) note also that there was no significant change in the frequency of visits to health care providers after the spray program. In other words, while the subjective reports suggest an increase in frequency of undesirable effects, the severity of the effects were not sufficient to cause the individuals to seek medical care. This pattern was also noted in the study by Aer'aqua Medicine Ltd. (2001) in which most of the individuals reporting adverse effects did not seek medical attention.

Although Petrie et al. (2003) do not specify the application rate for Foray 48B , they indicate that the spray program in Auckland involved the control of the painted apple moth. The risk assessment for this program is available from the Auckland District Health Board (2002) and specifies an application of 5 L per hectare, identical to that used in the white-spotted tussock moth program in New Zealand (Aer'aqua Medicine Ltd. 2001). The Auckland District Health Board (2002) also specifies that the application rate corresponds to 500 mg Foray 48B per m^2 and that as many as 15 applications can be made to a single property, which brings the total application rate to as much as 75 L per hectare or 7.5 g Foray 48B per m^2. Petrie et al. (2003) do specify that their survey was conducted after three aerial sprays. While it is possilbe that other pesticides were applied in some areas over the course of this study, no information on such applications is discussed in Petrie et al. (2003). This study is discussed further in the dose-response assessment (Section 3.3.3).

Blackmore (2003) also compiled a self-reported series of incidents associated with effects in individuals living in the area studied by Petrie et al. (2003). This compilation appears to be an advocacy document from an organization called the "Society Targeting Overuse of Pesticides NZ" and does not attempt to provide any analysis or draw any conclusions on causality. Nonetheless, the information presented by Blackmore (2003) is generally consistent with the analysis presented by Petrie et al. (2003).

Other epidemiology reports involving exposure to *B.t.k.* are much less detailed, but they generally support those described above. In a study in which *B.t.k.* 3a3b was applied at a rate of $22 \cdot 10^6$ to $25 \cdot 10^6$ IU per hectare to control the spruce budworm, no medical problems were detected in a survey conducted among *B.t.k.* workers, 80 volunteers living in the treated area, and 80 controls living in an untreated area (Valero and Letarte 1989). Industrial reports also indicate that *B.t.k.* can be cultured from various superficial sites on exposed humans and that antibodies to *B.t.k.* are greater in individuals in areas sprayed with *B.t.k.* than in individuals in untreated areas (Abbott Labs 1992). No illnesses or infections attributed to *B.t.k.* were noted. The medical records of workers exposed to *B.t.k.* contained no references to ocular infection, soft tissue infection, or chronic respiratory infection attributable to *B.t.k.* (Abbott Labs 1992).

3.1.3. Mechanism of Action (Persistence and Pathogenicity)

While the mechanism of action of *B.t.k.* and other strains of *B.t.* is understood relatively well in target species (Section 4.1), there is little indication that *B.t.k.* or several other insecticidal strains of *B.t.* have any specific mechanism of action in humans or other vertebrate species (Addison 1995; Drobniewski 1994; McClintock et al. 1995b; Meadows 1993; Siegel et al. 1987; Siegel 2001).

Persistence refers to the ability of the organism to survive rather than multiply within a host. Several studies indicate that *B.t.k.* can be recovered from exposed mammals but that recovery decreases over time after exposure is terminated. *B.t.k.* and other strains of *B.t.* can be detected in experimental mammals several weeks after exposure (Oshodi and Macnaughtan 1990a,b,c; Siegel and Shadduck 1990; Tsai et al. 1995). Similarly, several of the epidemiology studies discussed in Section 3.1.2 (Cook 1994; Noble et al. 1992; Valadares de Amorim et al. 2001) report the recovery of *B.t.k.* from nasal swabs for up to several months after exposure—e.g., up to 120 days after workers applied *B.t.k.* (Cook 1994; Noble et al. 1992).

By definition, a pathogen will actively multiply in the host and cause damage. Various *Bacillus* species are clearly pathogenic to mammals (Drobniewski 1994). *B.t.k.* is clearly pathogenic to some insects including the gypsy moth but there is very little information suggesting that *B.t.k.* is pathogenic in other species.

Nonetheless, *B.t.k.* can cause toxicity in mammalian cell cultures *in vitro*. Tayabali and Seligy (2000) conducted numerous studies regarding the effects of a commercial formulation of *B.t.k.* (identified as F48B and presumably referring to Foray 48B) and subfractions of the formulation on human cell cultures. The cell culture endpoints examined were non-specific indices of cytotoxicity, including loss in bioreduction, morphological changes, changes in cell proteins, and cell breakdown (cytolysis). In addition, the cytotoxic effects of *B.t.k.* were compared to *B. cereus*. In general, the cytotoxic effects of *B.t.k.* were similar to those of *B. cereus* and could be blocked by antibiotics. In terms of the potential adverse human health effects *in vivo*, the authors note that *"... a sustained infection would be needed to generate sufficient amounts of vegetative cells and their cytolytic exoproducts"*.

The suggestion that *B.t.k.* may be pathogenic to humans (or other vertebrates) is limited to only one published study. Samples and Buettner (1983a,b) report that a farmer splashed a commercial formulation of *B.t.k.* (DiPel solution) in his right eye, causing eye irritation. Irrigation of the eye and application of an antibiotic ointment were ineffective in relieving the symptoms. Four days after the accident, the farmer was treated with 0.1% ophthalmic solution of dexamethasone, a corticosteroid given to relieve the irritation. A corneal ulcer was observed 10 days after the accident. The farmer was then treated with subconjunctival injections of antibiotics. *B.t.k.* was isolated and cultured from the ulcer. The farmer recovered with no permanent eye damage. Although this incident might be interpreted as evidence of an eye infected with *B.t.k.*, it can also be interpreted as severe eye irritation accompanied by the recovery of incidental, viable *B.t.k.* known to have been accidentally introduced into the farmer's eye (U.S. EPA 1986b). Other case reports of *B.t.* pathogenicity in humans involve strains other than *B.t.k.* (Siegel 2001).

Two studies have suggested that *B.t.k.* may contain diarrheal enterotoxins similar or identical to those in *B. cereus* (Damgaard 1995; Bishop et al. 1999). Damgaard (1995) used enzyme-linked immunosorbent analysis (ELISA), a very sensitive analytical method, and did detect enterotoxigenic activity in *B.t.k.* strain HD-1 as well as *B.t.k.* isolated from DiPel, Foray, and other formulations. The level of enterotoxigenic activity, however, was substantially less than

that of *B. cereus* (positive control): HD-1 11%, Dipel 0.8%, and Foray 3.4% [Damgaard 1995 Table 1, p. 247]. Also using an immunoassay, Bishop et al. (1999) detected diarrheal enterotoxins in *B.t.k.*. On the other hand, clinical signs of toxicity were not observed in rats at oral doses of 10^{12} spores per rat or subcutaneous doses of 10^{6} spores per rat. Fares and El-Sayed (1998) report that "*B.t.k.* HD-14" affects the gastrointestinal tract of mice. As discussed by Siegel (2001), however, the identification of HD-14 as *B.t.k.* may be incorrect. In any event, HD-14 is not present in commercial formulations of *B.t.k.* used in USDA programs to control the gypsy moth.

Some strains of *B.t.* produce a heat-stable substance commonly referred to as thuringiensin (U.S. EPA 1998). The beta-exotoxin is toxic to mammals and other non-target species (Section 4) and the mode of action involves the inhibition of RNA-polymerase (McClintock et al. 1995b). *B.t.k.* and other insecticidal strains of *B.t.* used in the United States do not contain a beta-exotoxin. Other strains of *B.t.* may contain a heat-labile alpha-exotoxin that causes effects similar to *B. cereus* (McClintock et al. 1995b).

Strains of *B.t.* are genetically similar to *Bacillus cereus*, a known human pathogen (Helgason et al. 2000). *B. cereus* was involved in cases of food-poisoning, causing both diarrhea and vomiting (Notermans and Batt 1998). Some strains of *B.t.*, not identified as *B.t.k.* , were implicated in episodes of gastroenteritis (Jackson et al. 1995). Furthermore, Vazquez-Padron et al. (2000) demonstrated that the Cry1Ac protoxin in *B.t.k.* strain HD-73 can bind to the gastrointestinal tract of mice, while Honda et al. (1991) demonstrated that the hemolysin in *B.t.k.* HD-1 is identical to the hemolysin produced by *B. cereus*. Hemolysin also was identified in several other strains of *B.t.* (Yang et al. 2003). Although Wencheng and Gaixin (1998) did not detect hemolysin in *B.t.k.* HD-1 or HD-73, hemolysin was detected in several other strains of *B.t.*

There is concern that different strains of *B.t.* may produce or acquire the capability to produce enterotoxins similar to those of *B. cereus*. Plasmid transfer between different species of *B.t.* under environmentally relevant conditions was demonstrated by Thomas et al. (2000). As discussed in the U.S. EPA (1998) RED for *B.t.* formulations, the transfer of diarrhoeal enterotoxins from *B. cereus* to various strains of *B.t.* is possible. Because of the relatively low incidence of food poisoning associated with *B. cereus* (i.e., about 0.64% of all cases of food poisoning), the lack of fatalities in cases of food poisoning associated with *B. cereus*, and the normal measures routinely taken to prevent all causes of food poisoning, the U.S. EPA (1998) does not consider the potential transfer to diarrhoeal enterotoxins from *B. cereus* to commercial strains of *B.t.* to be a substantial human health hazard.

Overall, the evidence for pathogenicity of *B.t.k.* is extremely limited. While the *in vitro* studies by Tayabali and Seligy (2000) clearly suggest that *B.t.k.* may damage cells in culture, the only *in vivo* study suggesting a infection in humans (Samples and Buettner 1983a,b) may reflect the persistence of *B.t.k.* rather than an infection. The human experience with *B.t.k.* is substantial, and, as summarized in Table 3-1 and discussed in Section 3.1.2, several epidemiology studies have looked for but failed to find evidence of *B.t.k.* pathogenicity in humans.

3.1.4. Acute Oral Toxicity

The U.S. EPA requires standard acute oral toxicity studies for the registration of most pesticides, including *B.t.k.* For microbial pesticides, an additional requirement includes assays for pathogenicity. The standard assays involving *B.t.k.* or its formulations are summarized in Appendix 1. The interpretation of these studies is reasonably unequivocal, suggesting that acute oral doses of *B.t.k.* or its formulations are essentially non-toxic and non-pathogenic (U.S.

EPA/OPP 1998). The same conclusion was reached by the World Health Organization (WHO 1999).

There is one controlled study in humans involving oral exposure to *B.t.k.*. Fisher and Rosner (1959) summarize a study in which 18 volunteers ingested a Thuricide formulation at a rate of 1000 mg per day for 5 days and were exposed to an inhalation dose of 100 mg per day (as a powder using an inhaler) for 5 days. No signs or symptoms of toxicity were reported and no changes in standard clinical tests of blood and urine were noted.

3.1.5. Subchronic or Chronic Systemic Toxic Effects

There are no recent studies regarding the subchronic or chronic toxicity of *B.t.k.* A standard 90-day subchronic feeding study and a 2-year chronic rat feeding study were conducted on an early commercial formulation of *B.t.k.* at a dose of 8400 mg/kg/day. No effects were seen in the 90-day study and the only effect noted in the 2-year study was a decrease in weight gain in female rats (McClintock et al. 1995b). Hadley et al. (1987) fed sheep (n=6 per group) two commercial formulations of *B.t.k.*, a Dipel formulation and Thuricide HP, for 5 months at a concentration of 500 mg per kg per day (corresponding to approximately 10^{12} spores per day). Loose stool or diarrhea was noted in some of the sheep consuming *B.t.k.* diets. This effect was not observed in untreated or vehicle controls. No other remarkable signs of toxicity were apparent. *B.t.k.* was detected in the rumen, blood, and some tissues of treated sheep.

3.1.6. Effects on Nervous System

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

Studies designed specifically to detect impairments in motor, sensory, or cognitive functions in animals or humans exposed *B.t.k.* or other strains of *B.t.* are not reported in the open literature or in the list of studies submitted to the U.S. EPA to support the registration and re-registration of *B.t.* Specifically, the U.S. EPA/OPTS (2003) has standard protocols for several types of neurotoxicity studies including a neurotoxicity screening battery (Guideline 870.6200), acute and 28-day delayed neurotoxicity of organophosphorus substances (Guideline 870.6100). Neither of these types of studies was conducted on any strain of *B.t.* Further, the RED for *B.t.* (U.S. EPA 1998) does not specifically discuss the potential for neurological effects.

As discussed in Section 3.1.2, a variety of effects characterized as neuropsychiatric—i.e., sleep disorder, difficulty in concentrating, and dizziness —are reported in the epidemiology study by Petrie et al. (2003). Consistent with the discussion presented by Petrie et al. (2003), these effects are most likely to reflect either anxiety or nuisance caused by aerial applications in general. Consequently, there is no indication that *B.t.k.* or other strains of *B.t.* are specific neurotoxins in humans or other mammalian species.

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 effects 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.

Neither the published literature nor CBI files provide any clear indication that *B.t.k.* will cause immune suppression. This is consistent with the assessment of the U.S. EPA (1998, p. 13): *No known toxins or metabolites of Bacillus thuringiensis have been identified to act as endocrine disrupters or immunotoxicants*. Based on studies of *B.t.i.* (*Bacillus thuringiensis israelensis*) in immune suppressed mice, WHO (1999) concluded that individuals with compromised immune systems are not at special risk from exposure to commercial formulations of *B.t.* (Section 6.1.7.2 of WHO 1999).

More recently, Hernandez et al. (2000) noted that a strain of *B.t.* was associated with increased mortality in mice treated with *B.t.* as well as an influenza virus. The strain of *B.t.* used by Hernandez et al. (2000) is identified as serotype 3a3b from Abbott Labs, identical to the active ingredient in an unspecified pesticide formulation. Serotype 3a3b3c is *B.t.k.* (Glare and O'Callaghan 2000, Table 2.1, p.2.1). Serotype 3a3b has been used to designate *B.t.k.*, but it can be applied to HD-1 or HD-73 (Hofte and Whiteley 1989, Table 4, p. 245). Thus, it is unclear whether the report from Hernandez et al. (2000) applies to *B.t.k.* HD-1. Moreover, it is not clear whether the mechanism of the increased mortality reflected immune suppression or a simple addition of stress to the animal. Nonetheless, the increase in mortality was dose-related in terms of the *B.t.* exposure combined with the influenza virus at 4% of the LD_{50} —i.e., 4 of 20 mice at 10^2 spores/mouse, 8 of 20 mice at 10^4 spores/mouse, and 14 of 20 mice at 10^7 spores/mouse with no mortality observed in the control group (0 of 20 mice) when mice were treated only with the influenza virus at 4% of the LD_{50} with no *B.t.* exposure. In addition, weight loss was observed in mice treated with influenza virus at 2% of the LD_{50} and this correlated well with the dose of *B.t.* 3a3b used to infect the mice suggesting that a low innoculum of *B.t.* was able to complicate an influenza virus respiratory tract infection in mice. No mortality was observed in any of the mice but there was a statistically significant decrease in body weight at 10^4 spores/mouse and 10^7 spores/mouse but not at 10^2 spores/mouse. Also, the observed partial protection to mice after use of a thuringolysin-specific monoclonal antibody suggests that additional *B.t.*-produced toxins such as phospholipase C and sphingomyelinase could be involved. Since treatment of mice with the influenza-virus infection inhibitor, amantadine, demonstrated that *B.t.* alone was not pathogenic, the authors speculated that the influenza virus may have transiently altered the function of the non-specific defense mechanisms of the respiratory tract – i.e., macrophages and other leukocytes – thus rendering the host susceptible to a pulmonary infection by a very low innoculum of *B.t.*

As detailed in Section 3.1.2, there is evidence that some workers may become sensitized to *B.t.k* (Bernstein et al. 1999; Laferriere et al. 1987). In addition to the possible development of sensitivity to *B.t.k.*, Swadener (1994) reports the following incident:

> *...during the 1992 Asian gypsy moth spray program in Oregon, a woman who was exposed to Foray 48B had a preexisting allergy to a carbohydrate that was present as an inert ingredient. Within 45 minutes of exposure, the woman suffered from joint pain and neurological symptoms.* (Swadener 1994, p. 16)

The description of this incident is attributed to a letter, dated August 12, 1992, from the Oregon Department of Human Resources to Martin Edwards of Novo Nordisk. In itself, this report

does not provide sufficient information to assess the credibility that the effect was associated with Foray 48B or to assess the seriousness of the reported effect. Although the Oregon Health Services (2003) *B.t.k.* fact sheet discusses the possibility that individuals may be allergic to components of the bacterial growth media in *B.t.k.* formulations, the incident summarized by Swadener (1994) is not mentioned.

3.1.8. Effects on Endocrine System

In terms of functional effects that have important public health implications, 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). Mechanistic assays are generally used to assess the potential for direct action on the endocrine system (Durkin and Diamond 2002). Neither *B.t.k.* nor any other strain of *B.t.* was tested for activity as an agonist or antagonist of the major hormone systems (e.g., estrogen, androgen, thyroid hormone). Accordingly, all inferences concerning the potential effect of *B.t.* on endocrine function must be based on inferences from standard toxicity studies. As noted in the previous section, U.S. EPA (1998) concludes that there is no basis for asserting that strains of *B.t.* are likely to have an impact on the endocrine system.

3.1.9. Reproductive and Teratogenic Effects

Specific tests regarding the effects of *B.t.k.* and other strains of *B.t.* on reproduction and development were not conducted and effects of that nature are not addressed specifically in the existing reviews or compendia on B.t.—e.g., Glare and O'Callaghan (2000), U.S. EPA (1998), WHO (1999). As with effects on the nervous, immune, and endocrine systems, there is no credible concern that *B.t.k.* or other strains of *B.t.* are to cause adverse effects on reproduction or developement in humans or other mammals.

As noted in Section 3.1.3.3, Petrie et al. (2003) surveyed birth outcomes before and after a Foray 48B spray program and noted no adverse effects. As discussed further in Section 4.1, the lack of adverse reproductive effects in mammals is supported in field studies conducted in areas treated with *B.t.k.*

3.1.10. Carcinogenicity and Mutagenicity

While the cancer risks of exposures to chemical carcinogens are relatively well characterized, carcinogenic and mutagenic effects are not typically associated with bacteria. As reviewed by McClintock et al. (1995b), *B.t.k.* was subject to a 2-year chronic dietary study in rats in which no effects were noted other than a decrease in weight gain among treated females. This is the kind of study typically conducted as an assay for potential carcinogenicity in mammals.

A formulation of *B.t.k.* (HD-1) from China was shown to cause a dose-related increase in chromatid and chromosome breaks in spermatogonia when injected into the abdomen of 5th instar grasshoppers (*Oxya chinensis*) (Ren et al. 2002). As discussed by Ren et al. (2002), this study may suggest a mechanism of action in insects. This study, however, does not suggest a potential human health risk.

3.1.11. Irritation (Effects on the Skin and Eyes)

As with acute oral toxicity, the U.S. EPA requires standard assays for dermal and eye irritation, and these studies are summarized in Appendix 1. While most studies indicate that *B.t.k.* is not a strong irritant to either the eyes or the skin, the study by Bassett and Watson (1999b) is somewhat unusual in that the erythema appears to be more pronounced than in most of the other studies. Moreover, in at least one animal, the erythema appears to have progressed rather than reversed over the 14-day post-observation period. Mild eye irritation is consistently seen

in studies involving exposure to Dipel (Kuhn 1999b) or Foray (Berg 1991a,b; Berg and Kiehr 1991).

As discussed further in the dose-response assessment, throat irritation in humans appears to be a plausible effect based on the epidemiology studies by Cook (1994) and Petrie et al. (2003). Furthermore, local inflammatory responses were observed in mice after intranasal instillations of *B.t.k.* (Hernandez et al. 2000).

The epidemiology study by Cook (1994) includes workers involved in both ground and aerial applications of *B.t.k.* During the ground application, the commercial formulation of *B.t.k.*, diluted with water, was delivered as a high pressure spray from high-lift units. Dilutions ranged from an initial 200:1 to 75:1. The decrease in the dilution rate was associated with the use of a finer spray. In the last spray cycle, a jet turbine aerosol generator (Rotomister) mounted on a trailer was used. Two contractor teams, designated **A** and **B**, were involved in the ground applications. A separate group of workers was involved in monitoring the effectiveness of the aerial application by the placement of cards used to measure droplet deposition. These individuals were generally exposed to air-delivered aerosol during the aerial application and for 2 hours or more after the application. In general, the workers did not wear protective equipment (e.g., goggles or face masks). Worker exposure was monitored by microbiological air sampling. Symptoms, including transient irritation of the eyes, nose, and throat, dry skin, and chapped lips, developed in approximately 63% of the workers, but in only 38% of the control group. No days of work loss were attributable to *B.t.k.* exposure. These data are discussed further in the dose-response assessment (Section 3.3).

Two other incidents involving eye irritation in humans after exposure to *B.t.k.* were reported in the literature (Green et al. 1990; Samples and Buettner 1983). The studies by Samples and Buettner (1983a,b) regarding the pathogenicity and persistence of *B.t.k.* is discussed in detail in Section 3.1.3. The report by Green et al. (1990) describes an incident in which a worker involved in the application of *B.t.k.* splashed the *B.t.k.* mixture in his face and eyes. The worker developed dermatitis, pruritus, burning, swelling, and erythema, with conjunctival irritation. A culture of the conjunctiva was positive for *B.t.k.* The worker was treated effectively with steroid cream applications to the eyelid and skin.

Ocular exposure to *B.t.k.* does not always result in serious eye irritation. Noble (1992) briefly summarizes an incident in which two individuals on bicycles were accidently sprayed in the face by ground spray workers. The face and eyes were washed immediately after the incident, and no residual eye irritation developed in either individual over a 21-day follow-up period. In a separate incident, two workers on the ground spray team in the British Columbia study were accidently sprayed in the face with the *B.t.k.* formulation. These workers experienced only slight redness of the eyes for several hours after exposure (Cook 1994). The ground spray workers in this study reported a higher rate of eye irritation, compared with the control population (Cook 1994).

In terms of the weight-of-evidence assessment, there seems to be little doubt that exposures to *B.t.k.* can result in irritation of the skin, eyes, and respiratory tract, all of which are demonstrated in animals studies as well as in epidemiology studies and case reports. Thus, all three irritant effects are rated with the highest possible score—i.e., I.A.1.a. As discussed further in the dose-response assessment and risk characterization, irritant effects are the most likely effects to result from general applications of *B.t.k.* over widespread areas.

3.1.12. Systemic Toxic Effects from Parenteral Exposure

Parenteral exposures involve injecting a substance into an animal, usually into a vein (i.v.) or into the abdominal cavity (i.p.). Several such studies were conducted on *B.t.k.* or *B.t.k.* formulations and these studies are summarized in Appendix 1. As discussed by McClintock et al. (1995b), these studies are used primarily as qualitative screening tools to assess pathogenicity and infectivity. In addition, these studies may be used to assess variations in toxicity among different commercial batches of *B.t.k.* formulations (e.g., Vlachos 1991) as well as differences in toxicity associated with different culture conditions (Siegel 2001). According to Siegel (2001), these tests may be most relevant to risk characterization in terms of comparing the toxicity of the microbial agent to known pathogens such as *B. anthracis*, which has an LD_{50} in mice of about 2.64 spores by intraperitoneal injection. As noted in Appendix 1, little or no mortality was observed in mice at intraperitoneal *B.t.k.* doses of up to 10^8 [one hundred million] cfu. Thus, relative to highly pathogenic bacteria, the apparent acute lethal potency of *B.t.k.* is extremely low.

3.1.13. Inhalation Exposure

Most of the studies summarized in Appendix 1 are reasonably consistent with the general assessment regarding the toxicology of *B.t.k.* formulations: irritant effects but no systemic toxic effects or infectivity. Two studies, however, are inconsistent with the other available information. In one of these studies, inhalation exposure of rats to very high levels of *B.t.k.* caused piloerection (an atypical condition in which the hair stands erect), lethargy, and frequent urination during exposure (Holbert 1991). Alopecia (hair loss) was observed in the rats several days after exposure. This study involved whole body exposures over a 4-hour period to a level of *B.t.k.* formulation (3.22 mg/L Foray 76B) that caused the rats to become coated with the test material. The investigators indicated that the hair loss was probably related to *B.t.k.* exposure. While the implications for human risk assessment, if any, are unclear, this is an unusual finding. The reason for the hair loss cannot be determined, and this effect is inconsistent with other studies on *B.t.k.*

Only two studies (David 1990c; Hernandez et al. 2000) have reported mortality after exposure to *B.t.k.* and both of these studies, while related to inhalation toxicity, involve atypical routes of exposure. Intratracheal instillations of bacteria are analogous to inhalation exposures in that the bacteria is essentially inserted into the lungs. One such study (David 1990c) was conducted on a *B.t.k.* Dipel formulation. As detailed in Appendix 1, toxic responses including death were observed in treated animals and the time-to-clearance (estimated from linear regression) was prolonged. Also, Hernandez et al. (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. The relevance of these two studies to the human health risk assessment is discussed further in Section 3.3 (Dose-Response Assessment).

3.1.14. Impurities

Any preparation of bacteria has the potential for contamination with other possibly pathogenic microorganisms, which presupposes the need for proper quality control procedures (Bernhard and Utz 1993). Between 1985 and 1987, random samples of *B.t.k.* purchased by the various states or provinces were found to contain various bacterial contaminants, although none was considered pathogenic. In response to the concerns raised by this contamination, manufacturers took steps in 1988 to ensure that each batch of *B.t.k.* is free of detectable levels of contaminants. Since 1988, no substantial levels of bacterial or yeast contaminants were found in *B.t.k.* samples (Reardon et al. 1994). As part of an epidemiology study conducted by Noble et al. (1992), Foray 48B samples were tested and found to contain no other bacteria.

U.S. EPA (1998) requires that spore preparations of *B.t.* are produced by pure culture fermentation procedures with adequate quality control measures to detect either contamination with other microorganisms or changes from the characteristics of the parent *B.t.* strain.

3.1.15. Inerts

Inerts are defined as compounds that do not have a direct toxic effect on the target species. Nonetheless, some inerts may be toxic to non-target species, including humans. For some chemicals, the presence of toxic inerts may be a substantial issue in a risk assessment. The minimal testing requirements for compounds that have been used as inerts or adjuvants for many years is a general problem in many pesticide risk assessments. For new inerts, the U.S. EPA does require more extensive testing (Levine 1996). U.S. EPA (2001) proposes to discontinue the use of the term *inerts* for the following reason:

> *Many consumers are mislead by the term "inert ingredient", believing it to mean "harmless." Since neither the federal law nor the regulations define the term "inert" on the basis of toxicity, hazard or risk to humans, non-target species, or the environment, it should not be assumed that all inert ingredients are non-toxic*. (U.S. EPA 2001).

Nonetheless, the term *inerts*, as defined above, is used widely in the literature regarding pesticides, including the current risk assessment. U.S. EPA (2001) classifies inerts into four lists: toxic inerts (List 1), potentially toxic inerts (List 2), inerts that cannot be classified because of limitations in the available data (List 3), and inerts that are nontoxic or generally recognized as safe (List 4).

The identity of some inerts in some formulations of *B.t.k.* are reported in the open literature, and this information is summarized in Table 3-2. As indicated in Table 3-2, most inerts identified in the open literature are classified as GRAS (generally recognized as safe) compounds and are approved for use as food additives (Clydesdale 1997). Two of the compounds listed in Table 3-2, methyl paraben and polyacrylic acid, are not approved as food additives and are classified as List 3 inerts in U.S. EPA (2001). Swadener (1994) raises concerns about many of the additives in Foray 48B, a *B.t.k.* formulation used in USDA programs, including those approved as food additives, and similar concerns are expressed by groups opposed to the use of *B.t.k.* formulations (e.g., http://www.vcn.bc.ca/stop/preface.html). For example, Swadener (1994) correctly notes that concentrated sodium hydroxide is a severe corrosive and can be extremely hazardous. This, however, is not germane to the hazard identification of Foray 48B or any other *B.t.k.* formulations. In these formulations, sodium hydroxide is used in relatively low concentrations. While the specific amount and function of sodium hydroxide cannot be publically disclosed, Clydesdale (1997) notes that sodium hydroxide is commonly used as a pH control agent. In this and other approved uses of sodium hydroxide as a food additive, sodium hydroxide is not likely to pose any risk whatsoever. In an aqueous solution such as a formulation of *B.t.k.*, sodium hydroxide (NaOH) will dissociate to the sodium cation (Na^+) and the hydroxide anion (^-OH), both of which are natural and essential components of all living organisms. Furthermore, Na^+ and ^-OH concentrations are highly regulated by normal biological processes.

Much more detailed information regarding the inerts in *B.t.k.* formulations and the manufacturing processes was obtained from the U.S. EPA in the preparation of this risk assessment (e.g., Berg et al. 1991; Birkhold 1999; Coddens 1990a; Coddens and Copper 1990; Eyal 1999; Jensen et al. 1990a,b,c,d,e; Hargrove 1990a,b,c; Knoll 1990a; Newton 1999; Rowell 2000; Sorensen et al. 1990a,b). These studies, which include details regarding the

product chemistry and manufacturing processes, are protected under FIFRA Section 12(a)(2)(D), therefore, cannot be released to the general public or summarized in any significant detail.

As noted in Table 2-1, Valent USA Corporation holds the current registrations for *B.t.k.* formulations. Nonetheless, some information is available in the open literature from previous registrants—i.e., Novo Nordisk (1993) and Abbott Labs (1992)—and this information remains relevant to the current risk assessments and can be disclosed. Novo Nordisk (1993) published a brief summary of the issues associated with the use of inerts in Foray 48B and the proprietary nature of inerts. Foray 48B is a mixture of *B.t.k.* and fermentation materials, which comprise almost 90% of the product. The added inerts (that is, those other than incidental fermentation products) include materials to inhibit the growth of bacterial or fungal contaminants. These additives are approved for use in foods in the United States and Canada. All of the Novo Nordisk inerts are on U.S. EPA List 3 or 4. No volatile solvents are used in Foray 48B. The Oregon Department of Human Resources reviewed the complete formulation in Foray 48B and determined that "... exposure to the ingredients in the Foray 48B formulation are unlikely to pose a public health threat to populations exposed to the spray in eradication programs" (Fleming 1993 p.1). More recently, Van Netten et al. (2000) analyzed the volatile components in Foray 48B and identified numerous organic compounds that are present in trace amounts. Many of these compounds are on the U.S. EPA List 3 or List 4. It is unclear which of these compounds are specifically added to the formulation (i.e., as inerts) and which compounds are by-products of the fermentation process used to produce Foray 48B.

Some additional information is also publically available regarding the manufacturing process for *B.t.k.* formulations. *B.t.k.* formulations are complex chemical mixtures. *B.t.k.* is cultured in large vats that contain, for the most part, water and nutrients. The nutrients consist primarily of sugars, starches, proteins, or amino acids. These nutrients are not added as pure and defined compounds but rather as chemically complex and variable biological materials such as animal foodstuffs, a variety of flours, yeasts, and molasses. Relatively small quantities of essential elements, minerals, or salts also may be added to create optimal growth conditions. Adjuvants, such as antifoaming agents, may also be used at various stages of production to enhance growth or facilitate the recovery of *B.t.k.* from the growth media. The other components of the formulation are mostly water and a complex mixture of culture media and metabolites. The composition used by a manufacturer may change over time, as different sources of nutrient material are used (Bernhard and Utz 1993).

As detailed further in the dose-response assessments for *B.t.k.*, the presence and identity of inerts, adjuvants, and contaminants in *B.t.k.* formulations has little impact on the dose-response assessment for potential human health effects (Section 3.3) or ecological effects (Section 4.3). In both cases, the available data are much better suited to a “whole mixture” risk assessment than a component based risk assessment. Thus, a component based assessment of each inert was not conducted because component based assessments for highly complex mixtures generally are not useful given that the uncertainty of a component based risk assessment increases as the number of components in a mixture increases (Mumtaz et al. 1994, U.S. EPA/ORD 2000). As recommended by U.S. EPA/ORD (2000), the risk assessment is based on the mixtures of concern, which, in this case, are the commercial formulations of *B.t.k.* The limitations and benefits of this approach are discussed further in the risk characterization (Section 4).

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview

Exposure assessments usually estimate the amount or concentration of an agent to which an individual or population might be exposed via ingestion, dermal contact, or inhalation. The exposure assessments are then compared with toxicity studies based on similar types of exposures—i.e., the dose-response assessment—and then the risk is quantified. The human health risk assessment for *B.t.k.* is unusual in two respects. First, as discussed in Section 3.1 (Hazard Identification) and discussed further in Section 3.3 (Dose-Response Assessment), 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. Second, the apparent lack of a specific mechanism of toxicity for *B.t.k.* makes selecting the most appropriate measure of exposure somewhat arbitrary.

3.2.2. General Issues

As discussed in Section 2 and considered further in Section 4.1, 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. Toxicity to sensitive insects like the gypsy moth is generally attributed to a combination of the delta-endotoxin and the spore coat. These two factors probably account for the potency of the commercial formulations in the bioassays used to determine the BIU/mg of commercial product. Unlike the gut of the gypsy moth, which has a high pH (that is, the gut is alkaline or basic) the stomach of most mammals, including humans, has a low pH (that is, the stomach contents are acidic). Thus, the delta-endotoxin is not toxicologically significant for humans.

Another commonly used measure of exposure to *B.t.k.* formulations is *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. In the case of monitoring *B.t.k.* formulations, some of the colonies will be *B.t.k.* and some colonies will be other endogenous bacteria. Microscopic examination, differential culturing, or other methods may be used to determine the number of colonies that are *B.t.k.* By this general method, the number of cfu per unit of surface area or volume of air, depending on the sampling method, may be determined. Each cfu can be formed from a droplet or particulate that contains one or more viable spores. Thus, the number of cfu per unit of surface area or volume of air does not correspond directly to the number of viable spores per unit of surface area or volume of air. Dilution methods can be used to determine the number of viable spores (Palmgren et al. 1986).

The significance of cfu as a measure of human exposure is limited. As discussed in Section 3.1.3, 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 toxicologically 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.

For example, assume that an aerial application of a *B.t.k.* formulation is made and that two air samples are taken, one immediately at the spray site and one upwind from the spray site. Droplets containing viable spores as well as other components in the *B.t.k.* formulation are

sampled at both sites for a fixed period of time. If the sample taken at the spray site yields 200 cfu and the sample upwind yields 20 cfu, it seems clear that the level of human exposure to the *B.t.k.* formulation at the upwind site is 10% of that directly beneath the spray. This is, however, only a conclusion regarding relative exposure to *B.t.k.* and implies nothing about its toxic potency. Accordingly, the number of cfu is used as a surrogate for exposure to the *B.t.k.* formulation.

As discussed below in Section 3.2.3 for workers and in Section 3.2.4 for members of the general public), data are available regarding cfu per volume of air (cfu/m^3) during application and for intervals up to several days after application. For such measurements, it is not reasonable to assume that cultured colonies represent exposure to the formulation. Some components in the formulation, like water or other volatile materials, will have evaporated, whereas other nonvolatile materials, like starches, sugars, minerals, proteins, and amino acids, will have degraded or partitioned from the viable spores. Thus, measurements of cfu taken long after the spray application can be interpreted as viable *B.t.k.* spores that probably adsorbed to particulates and were re-suspended.

Some of the available toxicity studies (Appendix 1) express exposure in units of mg of formulation per unit of body weight or volume of air, depending on the route of exposure. As with cfu, these measures may be applicable to the risk assessment in so far as the anticipated exposures involve the entire commercial formulation. Exposures of this nature usually occur during or immediately after application.

3.2.3. Workers

Studies that quantify exposures to workers (and members of the general public) are summarized in Table 3-3. No new worker exposure studies became available since the 1995 risk assessment. The two worker studies summarized in Table 3-3, Cook (1994) and Elliott et al. (1988), are identical to the studies used in the 1995 risk assessment.

In the study by Elliott et al. (1988), portable sampling pumps with 37-mm (0.8 micron pore size) cellulose ester membrane filters were used for personal and area air monitoring. Flow rates on the sampling pumps ranged from 0.1 to 2.0 L per minute, and the duration of sampling ranged from 0.25 to 4 hours. All personal monitoring done during 1986 was conducted with a flow rate of 0.1 L per minute. Microbial culture and microscopic examinations were used to assay for *B.t.* on the filter media. Initially, all plates (inoculated with membrane filters from the monitoring pumps) were incubated and inverted for 24 hours at 30°C, after which time colonies were counted. The plates were then incubated for 5 more days at room temperature. Colonies resembling *B.t.* were examined microscopically. *B.t.* was identified by the presence of diamond-shaped toxin crystals (Elliott et al. 1988). Measurements made during 1985 could not be expressed as cfu/m^3 because of the extreme numbers of colonies obtained on the culture plates. The results presented in Table 3-3 are based on 1986 monitoring of personal air.

Much higher exposure levels are reported in the study by Cook (1994). The substantial difference in exposure concentrations may be related to work practices and application methods,, which include ground applications in the study by Cook (1994) and aerial applications in the study by Elliott et al. (1988). In general, ground applicators are exposed to much higher concentrations of pesticides, compared with aerial applicators.

3.2.4. Members of the General Public

As noted in Section 2, *B.t.k.* as well as other strains of *B.t.* are naturally occurring bacteria. *B.t.k.* HD-1, the same strain used as a pesticide against the gypsy moth, is found in food as well as other environmental media (Damgaard et al. 1996; Damgaard et al. 1997b; Glare and O'Callaghan 2000).

In terms of exposure levels that can be meaningfully related to USDA program activities, the most appropriate measure of exposure with respect to workers is summarized in Table 3-3 in terms of cfu/m^3. The consistency among the various studies is noteworthy. During spray, members of the general public may be exposed to concentrations in the range of about 200 to 4000 cfu/m^3, which is about 2 to 3 times lower than of the range of exposure levels for workers involved in aerial applications— i.e., about 400 to 11,000 cfu/m^3— but very far below the exposure levels that Cook (1994) observed in ground workers (Table 3-3).

After spray, *B.t.k.* and the formulation products will disperse depending on wind speed and deposition. Teschke et al. (2001) note that concentrations in outdoor air may decrease by a factor of about 10 within 5 to 6 hours after spraying but that concentrations in indoor air may remain higher than those in outdoor air, probably due to decreased dissipation.

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview

In some respects, the dose-response assessment of *B.t.k.* is relatively simple. There is no information from epidemiology studies or studies in experimental mammals to indicate that *B.t.k.* will cause severe adverse health effects in humans under any set of plausible exposure conditions. This is also the conclusion reached by the U.S. EPA and the World Health Organization. The only human health effects likely to be observed after exposure to *B.t.k.* involve irritation of the skin, eyes, or respiratory tract.

Nonetheless, a recent epidemiology study suggests that the irritant effects of *B.t.k.* may occur with notable frequency at exposure levels typical of those used in programs to control the gypsy moth. On the other hand, a worker study indicates that the frequency of observing these irritant effects does not appear to increase substantially even at extremely high levels of exposure. The lack of a strong dose-response relationship is somewhat unusual but is consistent with experimental data in mammals.

From recent experimental studies not typically used in a quantitative dose-response assessment, it is possible to define extremely high exposures for *B.t.k.* that might pose a serious health hazard and it is possible to define a NOAEL for such effects that is consistent with the available human studies. Specifically, cumulative exposures of up to 1.4×10^{10} cfu/m^3 × hour are not likely to result in adverse effects.

The same study that can be used to derive this NOAEL also suggests that pre-exposure to viral infections of the respiratory tract may substantially increase the risk of serious adverse effects, including mortality in experimental mammals. While the dose-response relationship can be defined for a very specific situation —i.e., exposure of mice to 4% of the LD_{50} of an influenza virus—these data cannot be applied directly and quantitatively to the human health risk assessment.

3.3.2. Existing Guidelines

Dose-response assessments for the systemic toxic effects of most pesticides are 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.

This approach, however, was not taken by the U.S. EPA in the re-registration eligibility decision (RED) document (U.S. EPA 1998) for *B.t.* Similarly, the World Health Organization declined to derive an acceptable daily intake (ADI) value, an estimate that is analogous to the RfD, for *B.t.* (WHO 1999). In both cases, the decision not to quantify the dose-response relationship appears to be based on the very low mammalian toxicity of *B.t.* and its formulations as well as the human experience with *B.t.* considered in these documents. Specifically, the U.S. EPA states:

> *...no known mammalian health effects have been demonstrated in any infectivity/pathogenicity study .... The sum total of all toxicology data submitted to the Agency complete with the lack of any reports of significant human health hazards of the various Bacillus thuringiensis strains allow the conclusion that all*

> *infectivity/pathogenicity studies normally required ... be waived in the future as long as product identity and manufacturing process testing data indicate there is no mammalian toxicity associated with the strain* (U.S. EPA, 1998, p. 11).
>
> *The application methods suggest that the potential for eye, dermal and inhalation exposure to mixers, loaders and applicators does exist. ... However, because of a lack of mammalian toxicity, the risk from occupational exposure is minimal ... the health risk* [to the general public] *is expected to be negligible due to: (1) The lack of toxicological concerns associated with Bacillus thuringiensis, and (2) Bacillus thuringiensis has been used as a pesticide for approximately 50 years with no known adverse effects* (U.S. EPA, 1998, p. 14).

The World Health Organization reaches a similar conclusion:

> *Owing to their specific mode of action, Bt products are unlikely to pose any hazard to humans or other vertebrates or to the great majority of non-target invertebrates provided that they are free from non-Bt microorganisms and biologically active products other than the ICPs* [insecticidal crystal proteins]. *Bt products may be safely used for the control of insect pests of agricultural and horticultural crops as well as forests* (WHO 1999, Section 1.7, not paginated).

In terms of the standard risk assessment paradigm—hazard identification, exposure assessment, dose-response assessment, and risk characterization— U.S. EPA (1998) and WHO (1999) reach essentially the same functional conclusion: since no hazard identification can be made for a clearly adverse effect, a formal dose-response assessment is not necessary.

The current risk assessment does not substantially disagree with the assessment in U.S. EPA (1998) and WHO (1999). The available data do not indicate that any serious adverse effects are likely to occur under plausible conditions of exposure. Notwithstanding this assertion, the failure to quantify risk has limitations. First, as noted in the Introduction (Section 1), this risk assessment of *B.t.k.* is accompanied by risk assessments on other agents used against the gypsy moth and the failure to quantify risk prevents an explicit comparison of risks that may be useful in risk management decisions. Second, additional studies were published since the risk assessments presented by U.S. EPA (1998) and the WHO (1999) which are potentially useful for expanding on the dose-response assessment. Last, substantial public concern is often expressed over widespread aerial applications of *B.t.k.* and these concerns may be more fully addressed with an aggressive interpretation of the data.

3.3.3. Human Data

The quantitative dose-response assessment in the previous USDA risk assessment of *B.t.k.* (Durkin 1994; USDA 1995) is based largely on the worker study by Cook (1994), and this study remains the most complete assessment of the effects of *B.t.k.* in workers. Cook (1994) provides data on the overall incidence of various health effects in workers, compared with a control group of individuals not involved in the application of *B.t.k.* These data are summarized in Table 3-4. Based on a comparison between the control group and the workers, the data demonstrate (using the Fisher exact test and a p-value of 0.05) 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). Moreover, the overall incidence of all symptoms combined was increased significantly among the workers, compared with the controls .

In dealing with multiple comparisons, however, the use of the standard *p*-value of 0.05 may overestimate the number of significant associations. For example, if 100 sets of comparisons are made within the same population—i.e., there are by definition no differences because there is only one population—some comparisons may appear to be statistically significant only because of random differences in the sampling. To address this issue, one standard approach is to divide the pre-determined significance level, typically taken as 0.05, by the number of comparisons being made. This is referred to as Bonferroni's correction (e.g., Curtin and Schulz 1998). Thus, in the study by Cook (1994), the seven effects (excluding all effects combined) would lead to an acceptance level for statistical significance of about 0.007 [p-value of $0.05 \div 7 = 0.00714$].

While it is beyond the scope of this risk assessment to discuss Bonferroni's correction in detail, it should be noted that Bonferroni's correction is conservative—i.e., it will reduce the number of false positive associations. In terms of a risk assessment, Bonferroni's correction may be viewed as anti-conservative in that the presence of a large number of trivial comparisons could obscure statistically and biologically significant results for a subset of important comparisons. Thus, as discussed by Perneger (1998), judgement and an assessment of biological plausibility must be exercised in the application of Bonferroni's correction. Specifically for this risk assessment of *B.t.k.*, these judgements are discussed further in Section 3.2.5). When Bonferroni's correction is applied to the data from Cook (1994) in Table 3-4, none of the effects are statistically significant at $p<0.007$; however, skin irritation ($p\approx0.0077$) and throat irritation ($p\approx0.0079$) are marginally significant.

Confidence in the biological and statistical significance of these effects would be enhanced if dose-related or at least exposure-related trends were demonstrated. Cook (1994) does not provide incidence data segregated by exposure levels. Nevertheless, as summarized in Table 3-5 and illustrated in Figure 3-1, Cook (1994) provides data on the number of symptoms per worker segregated into three exposure groups as well as categories based on the use of protective masks. The exposure groups are based on cumulative $cfu/m^3 \times$ hours over three ranges: <1 to 100, 100 to 300, and >300. The use of masks is simply characterized as none, occasional, or regular. If the *B.t.k.* exposure levels are related to the symptoms considered by Cook (1994) as specified in Table 3-4, one might expect to see a positive association with exposure and fewer symptoms in workers wearing protective masks. As illustrated in Figure 3-1, such associations are few within or among the variables. Cook (1994) does not provide information about the control group in terms of average number of symptoms per worker and this lack of information may obscure an association. On the other hand, based on the results presented in Table 3-4, which include the incidence of various effects in the control group, it is not clear that combining all effects as a measure of response is meaningful. In other words, if only dermal irritation and irritation to the throat are statistically significant effects, the lack of clear exposure-response pattens for all effects combined (significant effects as well as random effects) might be expected.

At least one of the more recent epidemiology studies may be useful in further assessing the report by Cook (1994). Since the publication of the previous risk assessment, a number of epidemiology studies were published (Table 3-1), most of which fail to note remarkable or statistically significant effects, like the epidemiology studies considered in the 1995 risk assessment (i.e., Elliott et al. 1988; Elliott 1986; Green et al. 1990; Noble et al. 1992).

Although some of the more recent studies are discussed further in the risk characterization (Section 3.4), the study by Petrie et al. (2003) is the only recent study that reports statistically significant effects.

As discussed (see Section 3.1.2), Petrie et al. (2003) surveys a group of individuals prior to a *B.t.k.* spray (n=292) and a subset of the group after a *B.t.k.* spray (n=181) recording their responses for 25 different endpoints. Based on the per cent responses reported in Table 1 of the study, Table 3-6 presents the number of responders with each effect before and after the spray operation. The statistical significance, using the Fisher Exact test is provided in the last column of Table 3-6.

The Petrie et al. (2003) study, like the Cook (1994) study, involves multiple comparisons. When the Bonferroni correction is applied to 25 comparisons, the adjusted p-value corresponding to 0.05 for a single comparison is 0.002 [0.05/25]. Based on this correction, only one endpoint, throat irritation, with a pair-wise p-value of 0.000048, is regarded as statistically significant. The interpretation of the respiratory effects observed in the study by Petrie et al. (2003) is less than straightforward because the effect could be due to or influenced by pollen count. As noted in the discussion by Petrie et al. (2003), pollen counts in Auckland peak from October to February. The pre-exposure survey was conducted at the end of October over a 10-week period prior to spraying, which started in January. The post-exposure survey was conducted at the end of March, about 12 weeks after the start of spraying. Consequently, portions of the pre-exposure and post-exposure periods and all of the spray period occurred during the pollen season. Since portions of the pre-spray and post-spray periods were concomitant with the pollen season, it is not clear whether this factor introduces a serious bias.

Nonetheless, both Cook (1994) and Petrie et al. (2003) report throat irritation as an effect in workers involved in the spray application of *B.t.k.* The effect is of marginal significance in Cook (1994) and of clear statistical significance in Petrie et al. (2003), using a *statistically* conservative correction for multiple comparison. This consistency combined with the animal data indicating that irritation of the mucus membranes of the throat and respiratory tract is a biologically plausible effect (see Section 3.1.13) suggests that these effects should be attributed to *B.t.k.* exposure.

As indicated in the exposure assessment (Table 3-3), workers in the study by Cook (1994) were exposed to concentrations of *B.t.k.* of up to 15.8×10^6 cfu/m^3 —i.e., about 16 million cfu/m^3. As indicated in Table 3-4, throat irritation was noted in 7% of the control group and 29% of workers applying *B.t.k.* Under the assumption of independence, the response associated with *B.t.k.* can be calculated using Abbott's correction:

$$\boldsymbol{P = (P^* - C) \div (1 - C)}$$

where ***P**** is the observed proportion responding, ***P*** is the proportion responding that can be attributed to exposure (in this case to *B.t.k.*) and ***C*** is the proportion responding in the control group (Finney 1972, p. 125). Using this correction, the estimated proportion of workers evidencing throat irritation attributable to *B.t.k.* exposure is about 0.24 [(0.29 - 0.07) ÷ (1 - 0.07) = 0.2366] or 24%.

Petrie et al. (2003) did not monitor *B.t.k.* concentrations in air. Based on monitoring data from similar applications (Table 3-3), members of the general public may be exposed to air concentrations ranging from approximately 100 to 4000 cfu/m^3 during or shortly after aerial applications of *B.t.k.* similar to those conducted in the study by Petrie et al. (2003). This range

is a factor of 3950 to 158,000 less than the 15.8×10^6 cfu/m^3 from the study by Cook (1994). In terms of the quantitative response for throat irritation, Petrie et al. (2003) report rates of 47÷292 (16%) in the pre-spray population and 58÷181 (32%) in the post-spray population. Again applying Abbott's correction, the estimated proportion of the population evidencing throat irritation attributable to *B.t.k.* exposure is about 0.19 [(0.32 - 0.16) ÷ (1 - 0.16) = 0.1904] or 19%. In that way, as with the number of symptoms per individual summarized in Table 3-5 and Figure 3-1 from the study by Cook (1994), there appears to be no dose-response relationship for throat irritation.

Two factors in the Petrie et al. (2003) study may obscure any underlying dose-response relationship. First, as noted above, the study was conducted during a period that overlapped with high pollen counts. Since the high pollen season encompassed the pre-spray and post-spray surveys, the extent of bias may not be substantial. The only way to have assessed this further would have been to include a non-exposed control population, which was not done in the Petrie et al. (2003) study. The other factor is the possible bias associated with the post-spray population. Only 181 of 292 (about 62%) of the individuals responding to the pre-spray survey responded in the post-spray survey. As noted by Petrie et al. (2003), it is reasonable to presume that individuals who felt that they were affected by the spray would be more likely to respond in the post-spray survey, compared with individuals who felt that they were not affected. This possible source of bias could be further assessed by considering the pre-spray survey results only for those individuals responding to the post-spray survey. This information, however, is not provided in the Petrie et al. (2003) publication.

3.3.4. Animal Data

As noted in Section 3.1.13 and summarized in Appendix 1, there is essentially no information indicating that inhalation exposure to *B.t.k.* 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.

The only study that provides a clear dose-response relationship for exposure to *B.t.k.* involves intranasal instillations (Hernandez et al. 2000). In the Hernandez et al. (2000) study, groups of 20 mice were dosed at rates of 10^2, 10^4, and 10^7 cfu/mouse with or without doses of influenza virus at 4% of the LD_{50}. In mice not exposed to the influenza virus, the only effect noted was local inflamation. Hernandez et al. (2000) do not discuss dose-severity or dose-response patterns for the inflammation. In an earlier study, mortality increased to 80% after 24 hours in mice dosed at 10^8 cfu/mouse evidenced 80% mortality (Hernandez et al. 1999). No mortality was observed In mice exposed to the influenza virus alone at 4% of the LD_{50} or in mice exposed to *B.t.k.* alone at doses of 10^2, 10^4, and 10^7 cfu/mouse. In mice exposed to both the influenza virus at 4% of the LD_{50} along with *B.t.k.* at doses of 10^2, 10^4, and 10^7 cfu/mouse, mortality was 4 of 20, 8 of 20, and 14 of 20 (Hernandez et al. 2000).

The data from the Hernandez et al. (1999, 2000) studies are illustrated in Figure 3-2, where, mortality is plotted on the Y-axis and $\log_{10}$ dose of *B.t.k.* (cfu/mouse) is plotted on the X-axis. The solid circles represent mortality data from mice treated with influenza and *B.t.k.* The solid line represents the fit of the mortality data to the the probit model using the U.S. EPA Benchmark Dose Software (http://www.epa.gov/ncea/bmds_training/software/overp.htm). The curved dashed line represents the 95% upper limit on risk. The probit model satisfactorily fits the data ($p<0.0001$), and the lower limit on the benchmark dose, based on an extra risk of 0.1, is estimated as 30 cfu/mouse. Because only one dose for the mice not treated with influenza

virus yielded partial mortality, no formal statistical analyses of these data are conducted. These data are simply illustrated in Figure 3-2 and a straight line is drawn from the highest dose at which no mortality occurred to the 80% mortality rate at a dose of 10^8 cfu/mouse.

In terms of the human health risk assessment, these data 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 could be observed in animals with a compromised respiratory tract (i.e., because of viral infection) because of 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. Table 3-7 provides dose conversions that may be valuable in further exploring the useful of these data. In Table 3-7, the first column indicates the cfu/mouse from the studies by Hernandez et al. (1999, 2000)and the second column provides the estimated concentration of *B.t.k.* required to achieve the cfu/mouse dose in a 1-hour exposure. This value is calculated as cfu/mouse divided by the estimated breathing rate (m^3/hour) of a 20 g mouse.

The calculated concentrations in air from cfu/mouse may be extremely conservative in the assumption that all of the inhaled *B.t.k.* will be retained. Nonetheless, the study by Holbert (1991) noted no mortality but some signs of toxicity in mice after 4-hour inhalation exposures to Foray 76B at a concentration of 3.13×10^9 cfu per L. This concentration is equivalent to 3.13×10^{12} cfu/m^3. Adjusting for the 4-hour exposure, the concentration is about 1.3×10^{13} cfu/m^3 × hours [3.13×10^{12} cfu/m^3 × 4 hours], which is approximately 5.5 times less than the concentration associated with 80% lethality in mice exposed to *B.t.k.* via intranasal installation (Hernandez et al. 1999) and approximately 1.8 times greater than the highest concentration associated with inflamation. While this cannot be overly interpreted, the signs of toxicity but lack of mortality observed in the Holbert (1991) inhalation study do appear to be reasonably consistent with the conversion of cfu/mouse to cfu/m^3 × hours presented in Table 3-7.

The best approach for extrapolating from mice to humans is uncertain. Following the suggestion by Siegel (2001), dose in units of cfu/mouse are converted to an equivalent cfu per human by adjusting body weight—i.e., 70 kg÷0.02 kg. These values are given in the third column of Table 3-7. The equivalent concentration in air is then calculated as the cfu per human divided by the breathing rate (m^3/hour) of a human engaging in moderate physical activity, presented in the fourth column of Table 3-7.

As noted in Section 3.2.3, exposures over a wide range of *B.t.k.* concentrations in air are associated with respiratory irritation in humans. At the lower end of the exposure range, concentrations probably in the range of 100 to 4000 cfu/m^3 are associated with an increased incidence of throat irritation in members of the general population based on the epidemiology study by Petrie et al. (2003). Monitoring data reported by Teschke et al. (2001) suggest that concentrations in outdoor air after 5 to 6 hours would be about 10-fold lower but that concentrations in indoor air could be approximately 250 cfu/m^3 (see Table 3-3). At the upper range of exposure, *B.t.k.* concentrations of up to 15.8×10^6 cfu/m^3 are associated with throat irritation in workers (Cook 1994). Both studies report similar response rates: about 19% in the lower exposure for the general public and about 24% in the occupational exposures.

According, there is no clear or strong exposure-response relationship. Severe adverse effects are not reported in either study.

This pattern is consistent with the available toxicity data in mice. Over a broad range of intranasal doses—i.e., 100 to 100-million cfu/mouse— the only effects reported by Hernandez et al. (2000) involve inflammation. Based on the estimates of human equivalent cfu/m^3 × hour presented in Table 3-7, exposures ranging from approximately 100,000 (1×10^5) to approximately 10,000,000,000 (1×10^{10} or 10 billion) cfu/m^3 × hours are likely to result in local inflamation but not mortality.

The mouse studies were conducted at doses that are not likely to be encountered by members of the general public exposed to *B.t.k.* Consequently, the mouse data cannot be used directly to support the responses reported by Petrie et al. (2003). Nonetheless, the weight-of-evidence suggests that some members of the general public could experience respiratory irritation at *B.t.k.* concentrations ranging from 100 to 4000 cfu/m^3. The apparent lack of a strong dose-response relationship in humans is consistent with the wide dose range leading to local inflamation in mice.

Finally, the failure to note any severe adverse effects in humans exposed to *B.t.k.* concentrations of up to 15.8×10^6 cfu/m^3 (1.58×10^7 cfu/m^3) reported by Cook (1994) is also consistent with the available animal data suggesting that no mortality would be expected at concentration of up to 1.4×10^{10} cfu/m^3 × hours. In other words, a worker would need to be exposed to 1.58×10^7 cfu/m^3 for about 37 days to reach a cumulative dose of 1.4×10^{10} cfu/m^3 × hours [(1.4×10^{10} cfu/m^3 × hours) ÷ 1.58×10^7 cfu/m^3 = 886 hours or about 37 days]. The highest cumulative exposure reported by Cook (1994) is $>3\times10^8$ cfu/m^3 × hours, a factor of about 50 below the highest estimated non-lethal exposure of 1.4×10^{10} cfu/m^3 × hours base on the available data in experimental animals.

3.3.5. Values Used for Risk Characterization

In some respects, the dose-response assessment for *B.t.k.* is not much different from that of the previous risk assessment (Durkin 1994; USDA 1995). Under plausible conditions of exposure, there is no indication that *B.t.k.* will cause severe adverse effects and the most plausible effects are likely to involve irritation.

The current dose-response assessment can be elaborated in two ways. First, based on a consideration of the study by Hernandez et al. (2000) and the estimates of equivalent human exposures given in Table 3-7, it seems plausible that cumulative exposures up to 1.4×10^{10} cfu/m^3 × hour will not cause adverse effects. This assumption is based on the 1×10^7 cfu/mouse dose group in the study by Hernandez et al. (2000) in which local inflammation was the only adverse effect observed. Further support is drawn from the NOAEL of 3×10^8 cfu/m^3 × hours for adverse health effects in humans reported in the Cook (1994) study in which the only effects of marginal significance are throat irritation and skin irritation. The potential need for an uncertainty factor on the 1.4×10^{10} cfu/m^3 × hour is questionable given the reasonable consistency of the human data with the animal data. This issue is discussed further in Section 3.4 (Risk Characterization).

While a human NOAEL for serious signs of toxicity can be estimated, the NOAEL for irritant effects cannot be estimated. The data suggest that at low and plausible concentrations associated with the normal application of *B.t.k.*, irritant effects may be reported by a substantial number of individuals—i.e., about 20% of the population. Irritant effects will also be reported at much higher concentrations, although the incidence of the effects may not be substantially greater.

Another major difference between the previous dose-response assessment for *B.t.k.* (Durkin 1994; USDA 1995) and the current risk assessment is the identification in the current risk assessment of a potential concern for individuals with respiratory diseases such as influenza. As illustrated in Figure 3-2, the study by Hernandez et al. (2000) clearly suggests that otherwise non-lethal doses of *B.t.k.* can be associated with pronounced lethality in mice infected with otherwise non-lethal doses of influenza virus. Based on the probit model, a benchmark dose of 30 cfu/mouse can be calculated.

Concern for the report by Hernandez et al. (2000) is somewhat enhanced by an earlier study by Berg (1990) in which rats were given an intravenous dose of 1 mL Foray 48B. Histopathological findings in the liver and the reticuloendothelial system were attributed to a background infection. The pathology results, however, were more severe in the exposed group compared with the controls. This could suggest that the *B.t.k.* may have aggravated this disease condition. Most of the histopathological findings, however, appear to have been due to extensive removal of bacteria by the reticuloendothelial system, including Kupffer cells in the liver, spleen, and lymph nodes. Thus, this study may simply suggest that *B.t.k.* organisms can survive and reproduce in a mammalian host (i.e., persistence) rather than suggest any underlying pathogenicity.

It is unclear whether or not the data on mice exposed to both *B.t.k.* and an influenza virus can or should be applied directly and quantitatively to the human health risk assessment. One very significant problem in the quantitative use of these data is in the interpretation of 4% of the LD_{50} for mice relative to possible disease conditions in human populations. This issue is discussed further in the risk characterization.

3.4. RISK CHARACTERIZATION

3.4.1. Overview

The risk characterization for *B.t.k.* and its formulations is consistent with the risk characterization in 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 irritant effects to the skin, eyes, and respiratory tract; however, serious adverse health effects are not of plausible concern. Nevertheless, the approach used to quantify risk for irritant effects and more serious health effects is different, based on recent information regarding *B.t.k.* exposure.

Unlike the previous USDA risk assessment on *B.t.k.*, this document does not attempt to quantify the risk of irritant effects since there is no clear threshold for those effects. When *B.t.k.* is applied under conditions similar to those used in USDA programs to control or eradicate the gypsy moth, irritant effects are likely to occur in some members of the general public as well as in some workers. Throat irritation is the best documented health effect in humans after exposure to *B.t.k.*; however, skin irritation and eye irritation are also likely to occur, although perhaps at the upper extremes of exposure.

Although serious adverse health effects in humans are not likely to result from *B.t.k.* applications, this risk assessment, unlike the previous USDA risk assessment and the risk assessments conducted by the U.S. EPA and the World Health Organization, considers the possibility that serious adverse effects may result from exposure to *B.t.k.* and quantifies the risk. The bases for this approach are the recent *in vitro* studies suggesting that cellular damage is a plausible effect of *B.t.k.* exposure and the *in vivo* studies indicating that serious effects, including mortality, are possible at extremely high exposure levels. There is however, no reason to assume, given the reasonably good monitoring data, conservative exposure assumptions, and highly aggressive and conservative use of the available toxicity data, that any human population—ground workers, aerial workers, or members of the general public—are likely to experience overtly toxic effects from the normal use of *B.t.k.* in programs like those conducted by the USDA. At the extreme upper range for ground workers, exposure levels are estimated to 25 times lower than the functional human NOAEL. For members of the general public, exposurelevels are estimated to be approximately 28,000 to 4,000,000 [4 million] times lower than the functional human NOAEL.

The available toxicity data give no indication that subgroups of the general population are likely to be remarkably sensitive to *B.t.k.*. Two recent epidemiology studies have found that asthmatics are not likely to be adversely affected by aerial applications of *B.t.k.* On the other hand, there is one essentially anecdotal reference involving a severe allergy to a carbohydrate in a *B.t.k.* formulation which is not supported, however, in any of the published epidemiology studies. Nonetheless, *B.t.k.* formulations are complex mixtures and there is a possibility that certain individuals may be allergic to one or more of the components in the formulations, as acknowledged by a state health service.

An incidence in which mortality increased substantially in mice pre-treated with an influenza virus and exposed to various doses of *B.t.k.* raises concern regarding the susceptibility of individuals with influenza or other viral respiratory infections to *B.t.k.* toxicity. The viral enhancement of bacterial infections is not uncommon, and the enhancement of *B.t.k.* toxicity by a viral infection is not altogether surprising. Nonetheless, the relevance of this observation to public health cannot be assessed well at this time. Although the concurrence of viral enhancement and *B.t.k.* exposure are not reported in the available epidemiology studies, it is not clear that the studies would detect such an event or that the effect is of plausible concern at

the typical or even extreme exposure levels anticipated in gypsy moth control programs. The viral enhancement of *B.t.k.* toxicity is likely to be an area of further study in the coming years.

3.4.2. Irritant Effects

As discussed in the Hazard Identification (Section 3.1), *B.t.k.* formulations can be irritating to the skin, eyes, and respiratory tract. This conclusion is consistent with previous risk assessments of *B.t.k.* and other strains of *B.t.* (U.S. EPA 1998; WHO 1999). Moreover, most of the material safety data sheets for *B.t.k.* include warnings about dermal, ocular, and respiratory tract irritation.

The extent to which these irritant effects are classified as *adverse* is largely semantic. Based on the available epidemiology studies (Table 3-2), these effects are not severe enough to compel the general public to seek medical attention or to cause individuals involved in the application of *B.t.k.* to lose time from work. Even so, among the adverse human health effects associated with *B.t.k.* exposure, irritant effects are the most common.

The principal issue in quantifying the risk for irritant effects in humans exposed to *B.t.k.* is the lack of a clearly defined threshold. As discussed in the dose-response assessment (see Section 3.3), throat irritation was reported by members of the general public after aerial applications of *B.t.k.* at rates typical of those used in USDA programs (Petrie et al. 2003). While a number of other adverse or at least undesirable effects also are noted by Petrie et al. (2003), the association of these effects with exposure to *B.t.k.* is less clear. For throat irritation, however, the association seems compelling (Table 3-6). In addition, workers reported throat irritation after exposure to higher levels of *B.t.k.* There does not appear to be a remarkable dose-response relationship for the incidence of throat irritation—i.e., about 19% in members of the general public at presumably low exposure levels and about 24% in workers at much higher concentrations.

The lack of a dose-response relationship raises questions concerning the biological significance of this effect, particularly at low exposure levels. As discussed by Petrie et al. (2003), there may be biases in an epidemiology study involving self-reporting that reflect anxiety rather than physical damage. Furthermore, as Petrie et al. (2003) indicate, their study was conducted during a period of high pollen counts, which may explain the apparent increase in throat irritation, assuming that the effect was confounded by allergies. Although a full study using a control population not exposed to *B.t.k.* might help to address the issue, both the pre-exposure and post-exposure periods covered by the study did partially encompass the pollen season. Supported by data on human exposure and the experimental studies in other mammals (see Section 3.1.11), the weight-of-evidence suggests that throat irritation reported by Petrie et al. (2003) may be biologically as well as statistically significant.

The inability to define a clear threshold for irritant effects and the lack of an apparent dose-response or dose-severity relationship substantially impairs the quantitative expression of risk based on the standard hazard quotient approach. For example, one approach to defining a pseudo-human NOAEL might be to assert that responders in the Petrie et al. (2003) study were probably exposed to higher concentrations of—i.e., greater than1000 cfu/m^3—and to propose that the lower range of plausible exposure —e.g., 100 cfu/m^3—might be used as a functional NOAEL for deriving hazard quotients. An approach analogous to this is taken in the previous USDA risk assessment of *B.t.k.* (Durkin 1994; USDA 1995).

The proposed approach is not taken in the current risk assessment because, in addition to the obvious problems with the logic of the approach and lack of data to support the presumed NOAEL, the resulting hazard quotients would be meaningless in terms of expressing risk. For

example, individuals exposed to 1000 cfu/m^3 would have a hazard quotient of 10 [1000 ÷ 100 cfu/m^3] and workers exposed to 15.8×10^6 cfu/m^3 (i.e., workers in the study by Cook 1994) would have a hazard quotient of 158,000 [15,800,000 ÷ 100 cfu/m^3], leading to the conclusion, based on the hazard quotients, that workers exposed to *B.t.k.* are at much greater risk than the general public to irritant effects, which is not the case, as noted in Section 3.3.3. Moreover, there is no evidence that a hazard quotient of 10 has any greater effect than hazard quotients of 10,000 or 100,000 or any lesser effect than a hazard quotient of 2.

Accordingly, the potential risks for irritation are not quantified in this risk assessment, and are addressed only qualitatively. As discussed in Section 3.3.3 (Dose-Response Assessment, Human Data), the studies by Cook (1994) and Petrie et al. (2003) provide credible evidence that some members of the general population and some workers may experience throat irritation after exposure to *B.t.k.* from aerial or ground applications. Irritation to the skin and eyes is also plausible, although less well supported by the available data in humans except under extreme exposure conditions.

Eye irritation may result when small amounts of commercial formulations of *B.t.k.* are splashed into the eyes. The probabilities of this event occurring under various exposure scenarios (that is, number of hours worked) cannot be estimated from available data. Nonetheless, there are reports of eye irritation resulting from direct splashing of *B.t.k.* formulations in the eye (i.e., Samples and Buettner 1983; Green et al. 1990). Thus, the probability of such an event seems sufficiently high to justify precautions when handling concentrated formulations in such a way that splashing into the eyes is not a potential risk. Also, workers exposed to *B.t.k.* may be at risk of skin irritation, and the study by Bernstien et al. (1999) suggests that skin sensitization is a plausible effect of exposure.

3.4.3. Serious Adverse Effects

The previous risk assessments on *B.t.k.*, including the previous risk assessment conducted for the USDA, accept the general premise that *B.t.k.* is essentially incapable of causing serious adverse health effects under any conditions (Durkin 1994; U.S. EPA 1998; USDA 1995; WHO 1999). More recent studies on *B.t.k.*, however, suggest that adverse effects are possible, albeit under extreme exposure conditions that are not representative of field applications of *B.t.k.* formulations. Tayabali and Seligy (2000) demonstrated that *B.t.k.* causes cytotoxicity *in vitro*. Also, as discussed in the dose-response assessment (see Section 3.3.4), the studies by Hernandez et al. (1999, 2000) allow for an estimate of lethal doses as well as doses in which no adverse effects, other than local inflamation, were noted.

The use of these data quantitatively in a risk assessment is admittedly tenuous. Nonetheless, as discussed in Section 3.3.4, these are the best data available. Although intranasal instillation is not a directly relevant route of exposure, the estimates of non-lethal and lethal concentrations are consistent with the *in vivo* inhalation study by Holbert (1991), and the estimated human NOAEL is consistent with the worker data from Cook (1994).

Based on the calculations summarized in Table 3-7, equivalent human exposure concentrations of 1×10^{10} cfu/m^3 × hour could be adopted directly as a NOAEL with a 10-fold higher dose [1×10^{11} cfu/m^3 × hour] as a LOAEL. As noted in Section 3.3, a case could be made for applying an uncertainty factor to the NOAEL. Typically, an uncertainty factor of 100 is used to account for species-to-species extrapolation or sensitive individuals. As detailed in Table 3-7, however, the very conservative approach used to the estimate the equivalent human concentration in air is less than that of the equivalent concentration for the mouse by a factor of more than 500. Thus, no additional uncertainty factor for the NOAEL of 1×10^{10} cfu/m^3 × hour

is used in this risk assessment. The potential for effects on sensitive individuals is discussed further in Section 3.4.3).

Using an approximated NOAEL of 1×10^{10} cfu/m^3 × hour for human exposure, the risk characterization for serious toxic effects is summarized in Table 3-8. As indicated in the first column, three groups of individuals are considered: members of the general public, workers involved in aerial applications of *B.t.k.*, and workers involved in ground applications of *B.t.k.* A plausible range of concentrations for each group is based on published studies detailed in Table 3-3. For members of the general public, the concentration ranges from 100 to 5000 cfu/m^3. The lower end of this range is somewhat higher than outdoor concentrations anticipated 5 to 6 hours after spraying (Teschke et al. 2001). The upper range is set to encompass the highest reported concentration—i.e., 4200 cfu/m^3 from Elliott et al. (1988). The concentrations for aerial workers are based on the study by Elliott et al. (1988), and the concentrations for ground workers are based on the study by Cook (1994). For members of the general public, the duration of exposure is taken as 24 hours. Based on the monitoring data by Teschke et al. (2001), this duration is likely to be extremely conservative but is intended to encompass the possibly higher concentrations of *B.t.k.* measured in indoor air relative to outdoor air 5 to 6 hours after application (Teschke et al. 2001). For workers, the duration of exposure is taken as 8 hours to account for a regular work day. Since workers are not likely to spend 8 hours applying *B.t.k.* due to other job requirements, this exposure duration is probably somewhat conservative. An additional ground worker group, labeled as *extreme range*, is added to account for the report in Cook (1994) that some ground workers may have been exposed to *B.t.k.* concentrations greater than 300 million cfu/m^3 × hour. The cumulative exposure is then calculated in the fourth column of Table 3-8 as the product of the concentration and duration of exposure—i.e., hours × cfu/m^3. The hazard quotient is given in the last column as the cumulative exposure divided by the estimated human NOAEL of 1×10^{10} cfu/m^3 × hour.

The interpretation of the hazard quotients is simple and unambiguous. Given the reasonably good monitoring data, conservative exposure assumptions, and aggressive and conservative use of the available toxicity data, there is no reason to assume that any member of the human population—ground workers, aerial workers, or members of the general public —are likely to experience overtly toxic effects from the normal use of *B.t.k.* in programs like those conducted by the USDA. The extreme upper range of exposure levels for ground workers are estimated to be below the functional human NOAEL by a factor of 25. For members of the general public, exposures are estimated to be below the functional human NOAEL by factors of about 28,000 to 4,000,000 [4 million].

These or any other numerical expressions of risk must be interpreted with some caution. In the recent review of the toxicity of several strains of *B.t.k.* to mammals, Siegel (2001) quotes an earlier assessment by Burges (1981) concerning general testing needs for microbial pesticides, and this quotation bears repeating:

> *... a "no risk" situation does not exist, certainly not with chemical pesticides and even with biological agents one cannot absolutely prove a negative. Registration of a chemical is essentially a statement of usage in which the risks are acceptable. The same must apply to biological agents.* – Burges (1981, pp. 738-739).

Within this definition of safety or acceptable risk, there remains no basis for asserting that the use of *B.t.k.* to control the gypsy moth is likely to have adverse toxic effects on any group.

A major and extremely important uncertainty in this risk characterization concerns the use of a toxicity study involving nasal instillation and the attendant uncertainties in extrapolating this type of study to inhalation exposures in humans. An inhalation study similar in general design to the study by Hernandez et al. (2000) – i.e., using mice challenged with an influenza virus as well as appropriate controls – would be necessary for assessing more fully and improving the quality of the risk characterization.

3.4.4. Groups at Special Risk

The previous USDA risk assessment (Durkin 1994; USDA 1995) notes a weakly positive relationship in the incidence of irritant effects in ground workers with and without a history of asthma, seasonal allergies, or eczema (Cook 1994). Swadener (1994) also notes that some formulations of *B.t.k.* contain sodium sulfite, which may cause adverse effects in asthmatics taking steroid treatments. As discussed in Section 3.1.2, Pearce et al. (2002) conducted an epidemiology study designed specifically to address the potential increased risk for young asthmatics exposed to *B.t.k.*. The results of the study indicate that there were no significant differences among individuals present inside or outside the treated area. The study, which involved subjective reports of health as well as clinical measurements of peak expiratory flow rates has limitations. Specifically, the treated and control areas were close to one another, and the monitoring data indicate that individuals in the treated and control areas were exposed to *B.t.k.* Nonetheless, there was no detectable adverse effects in either population (Pearce et al. 2002).

Swadener (1994) summarizes an incident in which a carbohydrate inert in Foray 48B may have caused an allergic response in one woman. As discussed in Section 3.1.7, the incident is not well documented and the interpretation remains uncertain. Commercial formulations of *B.t.k.* are complex mixtures of many different carbohydrates and other materials to which certain members of the general population may be allergic (Oregon Health Services 2003). There is, however, no documented case of a severe allergic response in the epidemiology studies conducted on *B.t.k.* (Table 3-1).

Hernandez et al. (2000) demonstrate a substantial increase in mortality in mice pre-treated with an influenza virus and exposed to various doses of *B.t.k.* The study raises concern regarding the susceptibility of individuals with influenza or other viral respiratory infections to the toxicity of *B.t.k.*. As illustrated in Figure 3-2, increased mortality was observed at a very low dose—i.e., 100 cfu/mouse —which is one-million times lower than the lethal dose in non-viral treated mice—i.e., 1×10^8 cfu/mice. Based on an extra risk of 0.1, the estimated lower limit on the benchmark dose is 30 cfu/mouse (see Section 3.3.4). Following the conversion approach used in Table 3-7, this value corresponds to a human exposure level of 42,000 cfu/m^3. The use of the LD_{10} is not to suggest that such a risk is acceptable but rather to illustrate an exposure level for which the response rate would be readily detected in most epidemiology studies.

The potential significance of the Hernandez et al. (2000) study to public health is difficult to assess. As noted in Table 3-3, most human exposure levels are well below 42,000 cfu/m^3. On the other hand, cumulative exposure levels for the general public, based on the conservative estimates used for this risk assessment, could range up to 360,000 cfu/m^3 × hours. More plausible estimates, based on only a 2-hour rather than a 24-hour duration, range from 1200 to 30,000 hours × cfu/m^3 for members of the general public. Consequently, it is not clear whether the human experience with *B.t.k.*—i.e., the epidemiology studies summarized in Table 3-3—can be used as evidence to preclude the possible association between viral infections and the enhanced toxicity of *B.t.k.* or to establish that the viral enhancement of *B.t.k.* toxicity is not of plausible concern regarding human exposure. Such effects were not observed in ground workers, who clearly are exposed to *B.t.k.* concentrations far greater than 42,000

cfu/m^3 × hours. Nonetheless, the viral enhancement of bacterial infections is not uncommon and the enhancement of *B.t.k.* toxicity by a viral infection seems plausible. This issue is likely to the subject of further study in the coming years and should be monitored by groups involved in the use of *B.t.k.*

3.4.5. Cumulative Effects and Connected Actions

The cumulative effects associated with the application of *B.t.k.* formulations must consider the normal background exposure to *B.t.k.*, residual exposure to *B.t.k.* and formulation products after a single application, and the effects of multiple applications in a single season and over several years. Since the dose-response assessment is based on measures of cumulative exposure —i.e., hours × cfu/m^3—and is supported by epidemiology studies, this type of cumulative effect is implicitly considered in the dose-response assessment. Given the reversible nature of the irritant effects of *B.t.k.* and the low risks for serious health effects, cumulative effects from spray programs conducted over several years are not expected.

Workers or members of the general public who are exposed to aerial or ground sprays of *B.t.k.* also will be exposed to the gypsy moth and may be exposed to other control agents. There are no data indicating that risks posed by these other agents will affect the response, if any, to *B.t.k.* formulations. Similarly, exposure to other chemicals in the environment may impact the sensitivity of individuals to *B.t.k.* or other agents; however, the available data are not useful for assessing the significance of such interactions.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview.

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 persistent in mammals for several weeks after exposure, there is little indication that oral or dermal exposure leads to any 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 exposed to applications of *B.t.k.* Nonetheless, there are data to suggest that extremely high concentrations of *B.t.k.* in air might pose a hazard.

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 per kg bw or at multiple oral doses up to 2857 mg formulation per 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. The apparent lack of *B.t.k.* toxicity is supported by numerous field studies in birds. In one field study, a transient decrease in abundance was noted in 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 germinate in the intestinal tract and enter the body cavity through the perforations made by the crystal toxins. The bacteria replicate in the body cavity, causing septicemia and eventual death. While various strains of *B.t.* are often characterized as selective pesticides, *B.t.k.* is toxic to several species of target and non-target lepidoptera. Sensitive non-target 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 non-target 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 *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.

U.S. EPA (1998) raises 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 process. 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. Toxicity to Terrestrial Organisms.

4.1.2.1. Mammals –The hazard identification for mammals is closely related to the hazard identification for the human health risk assessment (see Section 3.1) in that both are based, in part, on numerous standard toxicity studies in experimental mammals (Appendix 1). As discussed in Section 3.1 and summarized inAppendix 1, *B.t.k.* may persistent—i.e., may survive and be recovered—in mammals for several weeks after exposure; however, there is little indication that oral or dermal exposure leads to serious adverse health 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 in which no adverse effects were observed in populations of mammals exposed to *B.t.k.* applications of (Belloq et al. 1992; Innes and Bendell 1989). Nonetheless, as discussed in the human health risk assessment (see Section 3.3.4), there are data to suggest that extremely high air concentrations of *B.t.k.* in air might pose a hazard.

Acute oral doses of up to approximately 5000 mg per bw of *B.t.k.* formulations do not cause adverse effects in rodents (Bassett and Watson 1999a; Kuhn 1998b; Cuthbert and Jackson 1991; Kuhn 1991). Other acute oral toxicity studies report exposure levels in units of cfu per rat and indicate that doses of up to 10^8 cfu per rat are not associated with signs of toxicity (David 1990b; Harde 1990b). Similarly, in longer-term studies, *B.t.k.* doses of up to 8400 mg/kg/day were not associated with adverse effects in rats over a 2-year period (McClintock et al. 1995b) and doses of up to 500 mg/kg/day *B.t.k.* (corresponding to approximately 10^{12} spores per day) were not associated with adverse effects in sheep over a 5-month exposure period (Hadley et al. 1987). The only suggestion of an adverse effect is the death of one of four male Sprague-Dawley rats 1 day after a gavage dose of 5050 mg DiPel technical powder per kg. This effect, however, was attributed to a gavage dosing error that resulted in the accidental aspiration of the test material —i.e., inadvertently transporting the material into the lungs (Bassett and Watson 1999a). Thus, as in the human health risk assessment, the hazard identification for the oral route of exposure is essentially negative—i.e., there is no indication that adverse effects will result from oral exposure to *B.t.k.* or *B.t.k.* formulations at concentrations far higher than exposure levels which might be anticipated in the environment. Although the available studies report very high NOAELs, no LOAELs are reported.

Similarly, there is no indication that dermal exposures will result in adverse systemic effects. As summarized in Appendix 1, dermal applications of undiluted *B.t.k.* formulations will lead to irritant effects in rats and rabbits; however, no signs of systemic toxicity—i.e., effects other than those at the site of application—are reported in the literature (Kuhn 1998b; Kuhn 1999a; Meher et al. 2002; Bassett and Watson 1999b; Jacobsen 1993; Berg et al. 1991; Kiehr 1991a).

Unlike oral or dermal exposure to *B.t.k.*, there is probable concern that extreme inhalation exposures may pose a risk of adverse health effects. As discussed in Section 3.1.13, this assessment is based on the studies by David (1990c) and Hernandez et al. (2000) indicating that intratracheal instillations and intranasal instillations, respectively, may lead to mortality in rats. Concern regarding the possible risk posed by inhalation exposure to *B.t.k.* is enhanced by reports of less severe adverse effects in rats (Holbert 1991, Appendix 1) as well as the report by Bassett and Watson (1999a), discussed above, indicating that accidental aspiration of a *B.t.k.* powder might have caused death in a rat. As discussed further in the dose-response assessment (Section 4.3) and risk characterization (Section 4.4), this information leads to the same assessment of risk as for oral and dermal exposures—i.e., the risk at environmentally plausible concentrations is very low. Unlike the case with either oral or dermal exposures, however, a LOAEL for serious toxic effects can be approximated for inhalation exposures.

4.1.2.2. Birds – 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 (Beavers et al. 1988a) or five daily-dose gavage administrations (Beavers 1991b; Lattin et al. 1990a,b,c,d,e,f,g), 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 (Appendix 2). Due to the lack of evidence regarding acute toxicity in birds exposed to *B.t.k.* formulations or other *B.t.* strains, the U.S. EPA did not require chronic or reproductive toxicity studies in birds.

The apparent lack of *B.t.k.* toxicity to birds is supported by several field studies summarized in Appendix 2. *B.t.k.* applied at rates sufficient to decrease the number of caterpillars had no substantial adverse effects on most bird species (Rodenhouse and Holmes 1992; Nagy and Smith 1997; Sopuck et al. 2002). The relatively minor effects observed in some species were considered indirect and attributed to alterations in the availability of prey rather than to the direct toxicity of *B.t.k.* (Gaddis 1987; Gaddis and Corkran 1986; Norton et al. 2001).

Sopuck et al. (2002) report an unusual observation regarding effects in songbirds exposed to *B.t.k.* As summarized in Appendix 2, these investigators conducted population surveys of 42 species of songbirds in areas treated with three applications of Foray 48B at a rate of 50 BIU/ha (approximately 20 BIU/acre). Significant effects were noted in only one species, the spotted towhee (*Pipilo maculatus*); however, the effect (a decrease in abundance) was noted only during the spray year and not 1year after treatment. As discussed by Sopuck et al. (2002), the reason(s) for this decrease are not apparent; however, the time course of the effect was not related to a decrease in caterpillar abundance. The authors suggest that the effect might be an artifact of using only a single pre-application survey. Generally, this study is consistent with other field studies indicating no substantial effects on bird populations exposed to *B.t.k.*

4.1.2.3. Terrestrial Invertebrates

4.1.2.3.1. Lepidoptera – The mechanism of action of *B.t.k.* in lepidoptera is relatively well characterized. *B.t.k.* vegetative cells produce spores and crystals. The crystals are repeating protein subunits composed of proteinaceous toxins, enzymes, and other proteins. *B.t.k.* must be eaten in order to be effective as an insecticide. The crystals dissolve in insect gastrointestinal tracts that have a high pH—i.e., they are alkaline or basic. Proteolytic enzymes in the insect gut and in the crystals themselves break down the crystals (prototoxins) into active toxic subunits. The toxins attach to the lining of the mid-gut of the insect and rupture the cell walls, which allows the alkaline contents of the gut to spill into the body cavity (Drobniewski 1994). The *B.t.k.* spores germinate in the intestinal tract and enter the body cavity through the perforations made by the crystal toxins, replicate, and cause septicemia. The body tissues of

the insect are consumed by *B.t.k.* The infected insect usually stops feeding within 1 hour (Abbott Labs 1992).

While strains of *B.t.* are often characterized as selective pesticides (e.g., Paulus et al. 1999), various strains of *B.t.* are active in a large number of lepidopterans (e.g., Peacock et al. 1998) and are used to control of a variety of lepidopteran pests: spruce budworm (*Choristoneura fumiferana*), eastern hemlock looper (*Lambdina fiscellaria*), the diamondback moth (Perez et al. 1997a,b) et al. (Addison and Holmes 1996; Cooke and Regniere 1999; Gloriana et al. 2001; Masse et al. 2000). The insecticidal potency of *B.t.* varies depending on the strain of bacteria and type of insect (Frankenhuyszen et al. 1992, Navon 1993; Peacock et al. 1998).

Appendix 3 summarizes studies regarding the effects of *B.t.k.* on lepidopteran species. This appendix represents a subset of the most relevant available literature and is not comprehensive. As reviewed by Glare and O'Callaghan (2000), there are approximately 1500 reports that assay the effect of *B.t.k.* in different lepidopteran species. Some studies, like Miller (1990b) assay effects as changes in species abundance in non-target lepidoptera after applications of *B.t.k.* to control a pest species. In terms of the ability to characterize risk, however, this risk assessment focuses on studies that are useful for quantifying effects on non-target lepidoptera as well as differences in sensitivity among various species of non-target lepidoptera.

Herms et al. (1997) demonstrate the only dose-response relationships after applications of *B.t.k.* to both target and non-target lepidoptera. 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 to 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 to 37 BIU/ha treatment rate, and 14% at the 90 BIU treatment rate. Survival rates for gypsy moth larvae were: 80% for controls; 33% for low-dose treatment, and 5% for high-dose treatment. As detailed further in the dose-response assessment (Section 4.3), the differences between the gypsy moth and Karner blue do not appear to be substantial and the Karner appears to be as sensitive as the target species to *B.t.k.*

The sensitivities of larvae of two species of swallowtail butterflies (*Papilio glaucus* and *Papilio canadensis*) and the promethea moth (*Callosamia promethea*) also appear to be similar to that of the gypsy moth (Johnson et al. 1995). In the study by Johnson et al. (1995), several different types of trees (amalanchier, balsam poplar, black cherry, quaking aspen, and white ash) at several locations were treated with Foray 48B by backpack at a rate of 40 BIU/ha. On the day of treatment or 1 day after treatment, 1st and 2nd instar larvae of the test species were placed on foliage of the treated trees or untreated trees and mortality was monitored daily for 7 to 8 days. Given this experimental design, mortality could have occurred due to *B.t.k.* spray, natural causes, or predation. No significant differences were observed in mortality among the different types of vegetation but mortality was significantly and consistently greater on *B.t.k.* treated trees compared with untreated trees. Overall, survival after 8 days was about 30% to 40% in untreated trees and only 6% to 11% in treated trees (Johnson et al. 1995, Table 1, p. 292). Consistent with many other studies —see the review by Glare and O'Callaghan (2000)— mortality rates tended to be greater in shaded vegetation because of the longer persistence of *B.t.k.* In a separate series of studies with *Papilio glaucus*, significant mortality was noted when the larvae were placed on shaded vegetation for up to 30 days after the application of *B.t.k.* As

discussed by Johnson et al. (1995, p. 292), this is an unusual finding. In most other studies, the residual activity of *B.t.k.* ranges from about 2 to 10 days. One explanation for this effect offered by Johnson et al. (1995) is that the application by backpack may have resulted in coverage of both the top and bottom surfaces of the leaves thus increasing the functional persistence of *B.t.k.* on vegetation. Johnson et al. (1995, p. 294) also cite preliminary unpublished bioassay data from their laboratory indicating that swallowtail caterpillars may be over 100 times more sensitive than the gypsy moth to *B.t.k.* than the gypsy moth. In the absence of detailed data, this statement is difficult to evaluate. As discussed further in the dose-response assessment (Section 4.3), the survival rates reported by Johnson et al. (1995) are consistent with those in the gypsy moth and Karner blue from the study by study by Herms et al. (1997).

As noted above, Johnson et al. (1995) detected no significant differences in the toxicity of *B.t.k.* among different types of vegetation. In the forest tent caterpillar (*Malacosoma disstria*), a remarkably different pattern is observed with the target species apparently 100 times more sensitive to *B.t.k.* contaminated leaves from a secondary host, the sugar maple, compared with *B.t.k.* contaminated leaves from their primary host in north-eastern American, the quaking aspen (Kouassi et al. 2001).

James et al. (1993) assayed the toxicity of (Dipel-HG) to both the cinnabar moth (*Tyria jacobaeae*) larvae (1^{st} to 5^{th} instar), a non-target beneficial species, and the cabbage looper (*Trichoplusia ni*), a target species (1^{st} instars). This study involves the treatment of tansy ragwort, a pest weed that is consumed by the cinnabar moth, with various concentrations of *B.t.k.* equivalent to application rates of 2 to 250 BIU/ha. As summarized in Appendix 2 and discussed further in the dose-response assessment (Section 4.3), substantial differences were noted in sensitivity, with early instars of the cinnabar moth being relatively tolerant (LC_{50} values of 427 to 575 BIU/ha) and later instars being extremely sensitive (LC_{50} values of 19 and 26 BIU/ha). The sensitive instars are about as sensitive to the *B.t.k.* formulations as the target species (LC_{50} of 16 BIU/ha).

Not all non-target lepidoptera are as sensitive as the gypsy moth to B.t.k.. By far the most complete study regarding the toxicity of *B.t.k.* to non-target lepidoptera is the publication by Peacock et al. (1998). This investigators in this study used two formulations of *B.t.k.*, Foray 48B at a rate equivalent to 89 BIU/ha and Dipel 8AF at a rate equivalent to 99 BIU/ha. Foray 48B was assayed in 42 species from 7 families of lepidoptera and Dipel 8AF in 14 species from 4 families of lepidoptera. Various instars of larvae from each species were exposed to either control/untreated vegetation or vegetation treated with one of the formulations. Different bioassays used either *Carya ovata* (Shellbark hickory), *Juniperus virginiana* (Eastern red cedar), or *Quercus alba* (White oak). Larvae were placed on the treated vegetation, and mortality rates were observed for 5 to 7 days. Some bioassays using Foray were repeated in different years to assess variability in the potency of different batches of the formulation. The results of this study are summarized in Tables 4-1 (Foray formulation) and 4-2 (Dipel formulation). For both Foray and Dipel formulations, substantial differences in sensitivity among species and in some cases among families were noted. All species of Nymphalidae (n=3), Lasiocampidae (n=2), and Saturniidae (n=3) exhibited significant mortality in response to Foray. As in the study by Johnson et al. (1995), significant mortality was also observed in *Papilo glaucus* (Papilionidae). The largest number of species tested were from the Noctuidae (n=15), and significant mortality was established in only five species. Remarkably similar results were noted in all of the eight species tested with Foray using the same instar—i.e., the results were highly reproducible with little indication of substantial variability in the potency of different batches. The results with Dipel 8AF (Table 4-2) were similar to those with Foray 48B for nine species and different for only one species, *Eupsilia vinulenta*. This species appeared to

be sensitive to Foray 48B in two separate assays but insensitive to Dipel 8AF in one assay. This difference is noted by Peacock et al. (1998) but no explanation is offered. The only apparent difference in the two sets of bioassays is that the Foray assays were conducted on n-1/n–2 instars whereas the Dipel assay was conducted only on n–2 instars. Although the use of only one dose level for each formulation in the study by Peacock et al. (1998) precludes a direct dose-response assessment, these data can be used to bracket plausible ranges of sensitivity among non-target lepidoptera, as discussed further in Section 4.3.

The variability in the response of nontarget lepidoptera to *B.t.k.* is also illustrated in the recent field study by Rastall et al. (2003). In this study, a *B.t.k.* formulation (Foray 48F) was applied to two forests (dominated by oak, hickory, and maple trees) over a two year period at an application rate of 40 BIU/acre. This application rate is equivalent to about 99 BIU/ha, identical to the upper range of the application rate used in the bioassay study by Peacock et al. (1998). Rastall et al. (2003) monitored nontarget lepidopteran populations in the two years prior to application as well as over the two year period in which *B.t.k.* was applied. The response of nontarget lepidoptera varied substantially among different species. Larvae of three lepidopteran species were significantly decreased in treatment years: *Lambdina fervidaria* [geometrid], *Heterocampa guttivitta* [notodontid], and *Achatia distincta* [noctuid]. For 19 other species, larval counts were significantly higher in treatment years as were the total number of noctuids combined and the total number of all nontarget lepidopteran species combined.

4.1.2.3.2. Other Terrestrial Insects – Some non-target lepidopteran species may be as sensitive as target species to *B.t.k.*; however, most studies indicate that effects in other terrestrial insects are likely to be minor. As with the non-target lepidopteran species, there is a large body of literature available on other non-target insects. Most of the open literature is reviewed in Glare and O'Callaghan (2000), and much of the unpublished literature is reviewed in U.S. EPA (1998) and Abbott Labs (1992). This risk assessment focuses on those studies that suggest some plausible basis for concern in at least some species as well as those studies that can be used to quantitatively assess sensitivity relative to both target and non-target lepidoptera (Appendix 4).

There are no recent published or unpublished studies—i.e., since the preparation of the previous risk assessment for the USDA gypsy moth program (USDA 1995)—that report substantial effects in non-target insects, other than lepidoptera, exposed to *B.t.k.*. Wang et al. (2000) conducted a field study with Foray 47F on ants and noted no substantial effects on abundance and species richness, composition, or diversity over a 3-year post-application period. A slight decrease in abundance was noted in the third year of this study but was attributed to over-trapping. A substantial and significant decrease in collembolan populations was noted after the application of Dipel 8L that resulted in soil concentrations 1000 times greater than expected environmental concentrations (Addison and Holmes 1995). Dipel 4L is an oil-based formulation and the decrease in collembolan populations was also seen with the oil blank—i.e., the formulation inerts without *B.t.k.* Since the effect was not seen with Dipel 8 AF (which does not contain oil) or with unformulated *B.t.k.*, the effect on collembolan populations was attributed to the oil carrier rather than *B.t.k.* It should be noted that Dipel 4L is not used in USDA programs. As indicated in Section 2 (Program Description), only one oil-based formulation is used, Dipel ES, and no data regarding the toxicity of this formulation was encountered in the literature. As indicated in the risk characterization (Section 4.4), however, it is likely that any oil-based formulation could pose an increased risk to non-target species. Other recent studies on *B.t.k.* either report no effects in non-target species (e.g., Mohaghegh et

al. 2000) or are studies designed to assess the efficacy of *B.t.k.* in other pest species (Robacker et al. 1996).

One of the very few studies to report dose-related adverse effects in a non-target species is the early study by Haverty (1982). In this study, direct spray of lady beetles (*Hippodamia convergens*) and green lacewing (*Chrysopa carnea*) adults or larvae at rates equivalent to 79 and 158 BIU/ha resulted in slight but significant increases in mortality. Although this study also involved the use of Dipel 4L, mortality was not attributable solely to the oil carrier (Haverty 1982). As discussed further in the dose-response assessment, the rates of mortality observed in these species are consistent with those of *B.t.k.* in relatively tolerant non-target lepidoptera.

Honey bees are an important non-target insect for any pesticide, and bioassays on honey bees are required of all pesticides during the registration process. As noted by U.S. EPA (1998), the bioassays in honey bees submitted in support of the registration of *B.t.k.* suggest: "minimal toxicity for *B. thuringiensis* subspecies *kurstaki*" (U.S. EPA 1998, p. 21). This conclusion is also consistent with numerous laboratory bioassays and field studies concerning the effects of *B.t.k.* (Glare and O'Callaghan 2000; WHO 1999).

The current risk assessment does not substantially dispute these conclusions. Nonetheless, one of the studies cited by U.S. EPA (1998— i.e., Atkins 1991a cited as MRID 419835-01 on p. 19 of the EPA document) suggests that bees may be somewhat more sensitive than some non-target lepidoptera to *B.t.k.* exposure. In the study by Atkins (1991a), adult worker honey bees (*Apis mellifera*) were exposed to a dry flowable powder formulation of *B.t.k.* (14.52 BIU/lb) at deposition rates of 0 (control), 7.735, 15.470, and 23.205 µg/bee and these rates were equivalent to 0, 0.70, 1.4, and 2.1 lbs/acre. These application rates correspond to 0, 1.73, 3.45, or 5.19 lb/ha [1 acre = 0.4047 ha]. Given the potency of 14.52 BIU/lb, these application rates correspond to 25, 50, and 75 BIU/ha. As indicated in Appendix 4, these exposures resulted in mortality rates of 7.17 % (control), 18.96% (low exposure), 25% (mid exposure), and 24.91% (high exposure). As discussed in the dose-response assessment, these response rates are greater than the responses rates expected in relatively tolerant non-target lepidoptera.

4.1.2.3.3. Other Terrestrial Invertebrates – There is relatively little information regarding the toxicity of *B.t.k.* or its formulations to other terrestrial invertebrates. An early report by Benz and Altweg (1975) found no statistically significant effects (compared with water treated plots) on mixed earthworm populations over a period of about 8 weeks (May 5 to July 7) after the application of an older Dipel formulation (not otherwise specified) and a "Bactospeine" formulation of *B.t.k.* after soil applications equivalent to 1X, 10X, and 100X of the recommended application rates. Both Dipel 8AF (water-based formulation) and Dipel 8L (oil-based formulation) were tested at 1000X the expected environmental concentration (EEC)— i.e., 1.2 L/cm^3 in soil—by Addison and Holmes (1996) in a microcosm study using earthworms (*Dendrobaena octaedra*). Dipel 8AF caused no effect on earthworm populations over a 10-week observation period; however, Dipel 8L and the oil blank (i.e., the formulation without *B.t.k.*) caused decreased growth, greater than 50% mortality of the worms, and a decrease in the number of viable cocoons by week 6. Based on these results, Addison and Holmes (1996) further assayed Dipel 8L at 1X, 10X, 100X, and 1000X EEC. A significant reduction in survival, growth, and cocoon production was noted at 1000X EEC but no significant adverse effects on survival, growth, or reproduction were noted at 10X or 100X EEC. As discussed in Section 4.1.2.3.2 regarding effects on collembolan populations, the toxicity of Dipel 8L appeared to be related to the oil used in the formulation rather than to *B.t.k.*

4.1.2.4. Terrestrial Plants (Macrophytes) **–** As indicated in the re-registration eligibility document on *B.t.* (U.S. EPA 1998) , toxicity testing in non-target plant species was not required to support the re-registration of products containing *B.t.* because *"...a review of the literature on B. thuringiensis and its byproducts indicate no known detrimental effects on plant life..."*(U.S. EPA, 1998, p. 25). No information was found in the more recent literature regarding the toxicity of *B.t.k.* or its formulations to plants, suggesting that effects on plants are not likely and that the phytotoxicity of *B.t.k.* has not generated substantial interest. As reviewed by Glare and O'Callaghan (2000, p. 52), some lepidopteran species are used as biological control agents for weeds—such as the cinnabar moth (*Tyria jacobaeae*) to control ragweed. As discussed in Section 4.1.2.3.1 and detailed further in the dose-response assessment (Section 4.3), late instars of this species appear to be sensitive to *B.t.k.* and the use of *B.t.k.* could have secondary effects on the control of some weed species. It is likely, however, that the main impact of *B.t.k.* when used to control the gypsy moth will be in minimizing damage to terrestrial plants that would otherwise be damaged by gypsy moth infestations.

4.1.2.5. Terrestrial Microorganisms **–** There are relatively few studies regarding the effects of *B.t.k.* applications on terrestrial microorganisms. At exposure levels equivalent to 100X of the typical application rate for *B.t.k.* strain A20, Bernier et al. (1990) noted no effect on other soil microorganisms. At the recommended the rate, Dipel 176 (another oil-based formulation of *B.t.k.*) caused no effects on cellulose degradation, microbial biomass, or microbial respiration. At 1000X of the normal application rate, nitrite and ammonia metabolism were reduced and microbial biomass and respiration were increased after 8 weeks. As noted by Glare and O'Callaghan (2000), these effects could have been due either to *B.t.k.* germination or the effect of the oil in the formulation.

4.1.3. Aquatic Organisms.

4.1.3.1. Fish – As summarized in the previous USDA (1995) risk assessment on *B.t.k.*, field studies (Buckner et al., 1974; Otvos and Vanderveen 1993; Surgeoner and Farkas 1990) report no apparent fish kills or other adverse effects resulting from the use of *B.t.k.* Similarly, U.S. EPA (1998) classifies *B.t.k.* as virtually non-toxic to fish, based on an assessment of several acute toxicity studies in trout and one study in bluegills. These conclusions are consistent with a relatively large number of experimental studies that report very few if any effects in fish at much higher concentrations than would be encountered in the environment after the use of *B.t.k.* (Appendix 5). Acute exposure to *B.t.k.* formulations at concentrations up to 1000 mg/L are not associated with fish mortality (e.g., Meher et al. 2002), and longer-term studies of formulated *B.t.k.* in bluegills (Christensen 1990c), sheepshead minnow (Christensen 1991e) and trout (Christensen 1990d,i) report only decreased growth at concentrations up to 40,000X expected environmental concentrations.

The only suggestion of an adverse effect in fish is from the study by Martin et al. (1997). These investigators report an unexplained fish kill in Maryland after the application of *B.t.k.* In addition, these investigators conducted bioassays on Koi carp (*Cyprinus carpio*) at 1X and 10X ECC via food and water in experimental tanks for 32 days. The only adverse effects reported were changes in fish weight and plasma protein values. The Martin et al. (1997) report, however, is only an abstract and a full publication of this study was not found in the literature. Given the sparse detail in the abstract, it is difficult to interpret the significance of this study. No further information found regarding the fish kill purportedly associated with *B.t.k.,* and the information summarized in Appendix 5 as well as the information reported by Martin et al. (1997) do not support the contention that fish would be killed following the application of *B.t.k.*

4.1.3.2. Amphibians – There is available information regarding the toxicity of *B.t.k.* or *B.t.k.* formulations to amphibians. Other strains of *B.t.*, specifically *B.t. israelensis* and *B.t. tenebrions*, appear to have a very low toxicity to amphibians (Glare and O'Callaghan 2000; WHO 1999).

4.1.3.3. Aquatic Invertebrates – As summarized in Appendix 6, the effects of *B.t.k.* on aquatic invertebrates was investigated in both standard laboratory studies as well as a number of field studies. At concentrations sufficiently high to cause a decrease in dissolved oxygen or an increase in biological oxygen demand, *B.t.k.* exposure may be lethal to some aquatic invertebrates such as *Daphnia magna* (e.g., Christensen 1991d; Young 1990). Most organisms, however, seem relatively tolerant even to concentrations of *B.t.k.* in water that are up to 200,000 times higher than expected environmental concentrations (Christensen 1991f). Black fly larvae may be somewhat more sensitive than most other aquatic invertebrates to *B.t.k.* (Eidt 1985). Nevertheless, as discussed by Glare and O'Callaghan (2000), the different studies are difficult to compare with one another and some are difficult to relate to plausible environmental exposures because of different units in which exposures are expressed.

Several field studies (e.g. Kreutzweiser et al. 1992, 1993, 1994; Richardson and Perrin 1994) do not report remarkable effects in most species exposed to *B.t.k.* at levels that exceed expected environmental concentrations (EEC) by factors of up to 100. Possible exceptions may be stonefly larvae and mayfly larvae. Kreutzweiser et al. (1993, 1994) did note increased drift in decreased populations of stonefly larvae (*Leuctra tenuis*) at application rates equivalent to 10X EEC. After applications of *B.t.k.* at rates of 50 to 5000 BIU/ha over streams, Richardson and Perrin (1994) noted increased drift only in stonefly larvae.

U.S. EPA (1998) raises concerns that some batches of *B.t.* may contain heat labile exotoxins that are toxic to *Daphnia*. The production of these toxins is apparently not well understood and seems to be an atypical event probably associated with abnormal or poorly controlled production processes. U.S. EPA (1998) does not require daphnid testing of each commercial batch of *B.t.*; instead, the Agency requires manufacturers to submit a daphnid study on each new manufacturing process to demonstrate that heat labile exotoxin levels are controlled.

4.1.3.4. Aquatic Plants – The toxicity of *B.t.k.* to aquatic plants has not been tested because of the lack of information suggesting that adverse effects in aquatic plants are plausible (U.S. EPA 1998, p. 30). No relevant data that would call this judgement into question were found in the available literature.

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview.

The exposure assessment for the ecological risk assessment on *B.t.k.* are summarized in Table 4-3. Exposure assessments, based on the hazard identification, are presented for three groups: small mammals, terrestrial insects, and aquatic species. Although numerous exposure scenarios could be developed for terrestrial mammals, the only positive hazard identification for *B.t.k.* involves inhalation exposures. The ecological risk assessment uses inhalation exposure levels of 100 to 5000 cfu/m^3, which is the same range used in the human health risk assessment, 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 to 0.168 cfu/mouse. While there is no credible basis for asserting that terrestrial invertebrates are likely to have adverse effects after oral or dermal exposure to *B.t.k.*, 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. Consequently, the exposure assessment for this group is simply the range of application rates used in USDA programs—i.e., about 49 to 99 BIU/ha. For aquatic organisms, toxicity data are expressed in several different units, including 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 are expected to be less than or equal to 0.24 mg formulation/L. As discussed in the hazard identification, there is no basis for concern about adverse effects in birds, plants, soil microorganisms or invertebrates ,other than insects, exposed to *B.t.k.* Hence, explicit exposure assessments for these groups are not conducted.

4.2.2. Terrestrial Animals.

4.2.2.1. Terrestrial Vertebrates – Terrestrial animals might be exposed to any pesticide from direct spray, contact with contaminated media (vegetation, water, soil), the ingestion of contaminated media (vegetation, prey species, or water), or inhalation. Although numerous exposure scenarios could be developed for each of these types of exposure, the only positive hazard identification for *B.t.k.* involves inhalation exposures (see Section 4.1.2.1). As in the human health risk assessment (Section 3.4), inhalation exposures of 100 to 5000 cfu/m^3 are used to assess potential risks of serious adverse effects in terrestrial vertebrates.

The characterization of the potential risk from inhalation exposure is based on the cumulative exposure, which is expressed in units of cfu/organism, as in the human health risk assessment. The toxicity data are taken from laboratory studies involving *B.t.k.* exposure to mice (Hernandez et al. 1999, 2000). In terms of the exposure assessment, the mouse is an appropriate species on which to base the risk assessment because mice and other small mammals have a higher breathing rate per unit body weight, compared with larger animals. As noted in Table 3-7, the breathing rate for a 20 g mouse is approximately 0.0000014 m^3/hour. Taking the concentrations of 100 to 5000 cfu/m^3 and using a 24-hour exposure period (as in the human health risk assessment), the total cumulative exposure for a 20 g mouse ranges from 0.00336 to 0.168 cfu/mouse [100 to 5000 cfu/m^3 × 0.0000014 m^3/hour × 24 hours]. This cumulative exposure is used directly in the risk characterization (Section 4.4).

Although there is no credible evidence that oral or dermal exposure to *B.t.k.* is likely to cause adverse effects in terrestrial vertebrates, an extremely conservative exposure assessment for these routes of exposure can be developed. As noted in Section 4.1.2.1 and discussed further in the dose-response assessment (Section 4.3) and risk characterization (Section 4.4), free standing NOAELs are available for *B.t.k.* formulations in mammals, which are expressed in

units of mg formulation/kg body weight/day. The underlying assumption in this exposure scenario is that a small mammal consumes contaminated vegetation and contaminated water after having been sprayed directly with *B.t.k.* over its entire body surface.

The major routes of oral exposure are the consumption of contaminated vegetation and contaminated water. Initial residues on vegetation are determined by the type of vegetation and application rate. Fletcher et al. (1994) indicate that the highest residues are will be found on short grass—i.e., 240 mg/kg vegetation at an application rate of 1 lb/acre. As detailed in Table 2-1, the highest application for any *B.t.k.* formulation is 2 lbs/acre. Thus, the highest initial residues on vegetation are expected to be approximately 480 mg/kg on vegetation. General allometric relationships dictate that smaller animals, because of their higher metabolic rates, consume more food than do larger animals. Based on allometric relationships between food consumption and body weights for rodents (U.S. EPA/ORD 1993, p. 3-6), a small mammal weighing approximately 20 g will consume about 3.5 g of food per day. Thus, if a small mammal were to consume vegetation recently sprayed with a *B.t.k.* formulation, the dose to the animal would be about 84 mg/kg [0.480 mg/g vegetation × 3.5 g ÷ 0.02 kg].

An extremely conservative estimate of the dose from contaminated water can be derived in a similar way. Based on allometric relationships for mammals from U.S. EPA/ORD (1993, Eq. 3-17, p. 3-10), a small mammal will consume about 3 mL of water per day. As noted above, the highest application rate for any *B.t.k.* formulation is 2 lbs/acre, which corresponds to 224.2 mg/m^2. Under the assumption that the *B.t.k.* formulation is sprayed over a shallow (1 cm deep) puddle with a surface are of 1 square meter or 10,000 cm^2, the volume of water equals 10,000 mL and the initial concentration of the *B.t.k.* in the water is approximately 0.022 mg/mL [224.2 mg ÷ 10,000 mL]. Thus, the *B.t.k.* dose to the 20 g mammal is approximately 3.3 mg/kg [0.022 mg/mL × 3 mL ÷ 0.02 kg].

As a final component of this extreme exposure assessment, assume that the small mammal is sprayed directly with the *B.t.k.* formulation. Again using allometric relationships developed by U.S. EPA (U.S. EPA/ORD 1993, eq. 3-22, p. 3-14), a 20 g mammal has a surface area of about 0.0086509 m^2. Thus, at an application rate of 2 lbs/acre or 223.4 mg/m^2, the maximum dose that could be deposited on a 20 g mammal is about 97 mg/kg body weight [224.2 mg/m^2 × 0.0086509 m^2 ÷ 0.02 kg]. It is, of course, somewhat implausible to assume that the complete body surface will be covered by a direct spray; however, this calculation is maintained as an extremely conservative assumption. Furthermore, it is not reasonable to assume that the deposited dose will be absorbed. Nonetheless, one of the underlying assumptions for this conservative exposure assessment is that grooming by the small mammal results in the ingestion of the entire amount of *B.t.k.* formulation deposited on the mammal.

Combining these three routes of exposure, the total dose to the animal is approximately 184 mg/kg body weight [84 mg/kg + 3.3 mg/kg + 97 mg/kg = 184.3 mg/kg bw].

4.2.2.2. Terrestrial Invertebrates **–** As discussed in Section 4.1.2.3 (Hazard Identification for Terrestrial Invertebrates) and addressed further in Section 4.3 (Dose-Response Assessment), some terrestrial invertebrates, particularly lepidoptera, appear to be as sensitive to *B.t.k.* as the gypsy moth and other target species. While the dose-response assessment is somewhat elaborate, it is based on exposure units of BIU/acre or ha; thus, the exposure assessment is relatively simple—i.e., expressed in units of application rate. As indicated in Section 2.2, the application rates considered in this risk assessment are 20 to 40 BIU/acre, which are equivalent to about 49 to 99 BIU/ha.

A noteworthy reservation about using an application rate as a measure of exposure is that most of the toxicity studies do not involve field observations. Instead, different types of vegetation are treated in a manner equivalent to and expressed as an application rate, most often in units of BIU/ha. Thus, the effects of drift and canopy interception are not encompassed by the toxicity studies. This issue is addressed in the risk characterization (Section 4.4).

4.2.2.3. Other Terrestrial Species – As discussed in the hazard identification, there is no plausible basis for concern regarding adverse effects in birds (see Section 4.1.2.2), plants (see Section 4.1.2.4), soil microorganisms (see Section 4.1.2.5) or invertebrates other than insects (see Section 4.1.2.3.3) after exposure to *B.t.k.*. Thus, as with the previous USDA risk assessment (USDA 1995), explicit exposure assessments for these species are not conducted. The only reservation with this approach involves the used of oil-based formulations. This concern is addressed qualitatively in the risk characterization (Section 4.4).

4.2.3. Aquatic Organisms.

As illustrated in Appendix 5 (Toxicity to Fish) and Appendix 6 (Toxicity to Aquatic Invertebrates), toxicity data are expressed in several different units. Some field studies (e.g., Richardson and Perrin 1994), exposures are expressed application rates. Other studies report exposures as concentrations in units of mg formulation /L (e.g. Meher et al. 2002; Mayer and Ellersieck, 1986) and still other studies report exposures in units of cfu/L (e.g., Christensen 1990c,d) or IU/L (Eidt 1985). As noted by Glare and O'Callaghan (2000), this diversity of units impairs the ability to compare different studies. Nonetheless, as discussed further in the dose-response assessment (Section 4.4), the key toxicity values given in IU/L can be converted to units of mg formulation/L, which are the most useful units of measure for risk characterization.

The same approach can be used to derive conservative estimates of *B.t.k.* concentrations in water, expressed in units of mg of formulation/L, as was used to estimate exposure concentrations for a terrestrial mammal (see Section 4.2.2.1). For the mammal a depth of 1 cm was used to estimate an extreme worst-case concentration, which is not a reasonable assumption for exposure scenarios involving aquatic species. The U.S. EPA typically uses a water depth of 6 feet. Because of the apparently low potential for adverse effects, however, the U.S. EPA (1998) did not conduct an explicit exposure assessment on aquatic species. Most Forest Service risk assessments use a somewhat more conservative water depth of 1 m or about 3 feet, and this is the depth used to calculate a plausible concentration of *B.t.k.* formulation in water immediately after a direct spray of *B.t.k.* at an application rate of 2 lbs/acre or 224.2 mg/m^2. At a depth of 1 m, 244.2 mg of formulation would be deposited into 1 m^3 of water which is equivalent to 1000 L. Assuming instantaneous mixing, the concentration in water would be about 0.24 mg formulation/L [244.2 mg ÷ 1000 L].

For toxicity studies that are expressed in units of IU/L, the concentration of 0.24 mg formulation/L can be converted using IU/mg formulation values given in Table 2-1. The highest value is 32,000 IU/mg —reported for a number of formulations including Biobit HP, DiPel DF, and DiPel Pro DF. Thus, the concentration of 0.24 mg formulation/L corresponds to 7680 IU/L or 7.6 IU/mL [0.24 mg formulation/L × 32,000 IU/mg].

Some aquatic toxicity data are expressed in units of cfu/L, and these data cannot be converted readily to other units of exposure. Measurements of *B.t.k.* formulations are not expressed in units of cfu/mg formulation. Consequently, these units of measure are not relevant to those involved in the application of *B.t.k.* formulations. As an alternative, the monitoring study by Menon and De Mestral (1985) can be used to approximate plausible concentrations of *B.t.k.* in

water in terms of cfu/L. In this study, an older formulation of *B.t.k.*, Thuricide 16B, was applied at rates of 4.7 to 9.4 L/ha. Concentrations in river water ranged from 22 to 63 cfu/mL or 22,000 to 63,000 cfu/L. Menon and De Mestral (1985) do not report the potency of Thuricide 16B. Assuming that the nomenclature for Thuricide 16B is the same as that for the current Thuricide formulations, it is assumed that the Thuricide 16B formulation had a potency of 16 BIU/gallon. Thus, an application of 4.7 L/ha corresponds to application rate of approximately 8 BIU/acre [4.7 L/ha × 0.2642 gallon/L × 16 BIU/gallon × 0.4047 acres/ha = 8.0405 BIU/acre], and 9.4 L/ha corresponds to twice that amount or about 16 BIU/acre. It is not clear from the publication by Menon and De Mestral (1985) whether the reported cfu/L concentrations were associated with applications of 4.7 L/ha or 9.4 L/ha. For this component of the exposure assessment, it is assumed that the reported concentrations were associated with an application of 4.7 L/ha or 8 BIU/acre. In addition, the upper range of 63,000 cfu/L is used to calculate a water contamination rate of 7875 cfu/L per BIU/acre [63,000 cfu/L ÷ 8 BIU/acre]. As noted in Table 2-1, the maximum application rate of *B.t.k.* recommended for the control of the gypsy moth is 40 BIU/acre. Thus, the expected maximum concentration of *B.t.k.* in water is 3.15×10^5 cfu/L [7875 cfu/L per BIU/acre ×40 BIU/acre = 315,000 cfu/L].

Notice that this estimate of *B.t.k.* in water expressed as cfu/L is based on the most conservative set of assumptions from the study by Menon and De Mestral (1985) and may grossly overestimate actual exposure. The magnitude of the potential overestimation can be evaluated using the more recent monitoring study by Valadares de Amorin et al. (2001), in which *B.t.k.* concentrations in reservoirs were monitored after three applications of *B.t.k.* (Foray 48B) at a rate of 20 BIU/acre. The maximum number of *B.t.k.* colonies monitored by Valadares de Amorin et al. (2001) was 200 cfu/L (see Valadares de Amorin et al. 2001, Table 4, p. 1041).

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Overview.

The toxicity values used in the ecological risk assessment are summarized in Table 4-4. The dose-response assessment parallels the exposure assessment. Specific dose-response assessments are presented for three groups: small mammals, terrestrial insects, and aquatic species, both fish and aquatic invertebrates. For small mammals, dose-response assessments are given for inhalation and oral exposure. The risk assessment for inhalation exposure is based a study in which mortality increased in mice exposed to *B.t.k.* via intranasal instillations of the agent. A dose of 10^7 cfu/mouse is taken as the NOAEL, and 10^8 cfu/mouse is taken as a frank effect level—a dose associated with 80% mortality. The risk assessment for oral exposures is based on a free-standing NOAEL, which implies that oral exposure to *B.t.k.*, however high the concentration, will not cause adverse effects in mammals or birds. For this risk assessment, the dose of 8400 mg/kg/day is used as the NOAEL. For terrestrial invertebrates, sufficient data are available to estimate dose-response relationships for sensitive species and relatively tolerant species. Sensitive species, which consist largely of lepidoptera, have an LD_{50} value of about 21 BIU/ha. Tolerant species, comprised of some lepidoptera and other kinds of terrestrial insects, have an LD_{50} value of about 590 BIU/ha, which is approximately 28 times greater than the LD_{50} value for sensitive species, The dose-response curves developed for sensitive and tolerant species permit mortality estimates for any application rate. As with terrestrial insects, dose-response assessments are developed for tolerant and sensitive species of fish and aquatic invertebrates. Fish appear to somewhat less sensitive than invertebrates to *B.t.k.* exposure. For tolerant species of fish, the NOEC of 1000 mg/L, which corresponds to 2.5×10^{10} cfu/L, is taken from a study in mosquito fish. For sensitive species of fish, the LOEC is based on a trout study in which marginally significant mortality was observed at 1.4 mg/L or about 2.87×10^7 cfu/L. The most sensitive invertebrate species appears to be *Daphnia magna*, with a chronic NOEC of 0.45 mg/L or 6.24×10^8 cfu/L for both reproductive effects as well as mortality. The NOEC for tolerant species is taken as 36 mg/L based on bioassays in mayflies and caddisflies.

4.3.2. Toxicity to Terrestrial Organisms.

4.3.2.1. Terrestrial Vertebrates – As discussed in Section 4.2.2.1, two sets of exposure assessments are used for terrestrial vertebrates: inhalation exposures expressed in units of cfu/m^3 and oral exposures (including ingestion by grooming of material deposited on body surface) in units of mg formulation/kg body weight. These two types of exposures represent very different potential risks. More precisely, the assessment of the risk from inhalation exposure is based on the study by Hernandez et al. (2000) in which mortality in mice was observed after intranasal instillations of *B.t.k.* The assessment of oral exposures, on the other hand, is based on a free-standing NOAEL.

As discussed in Section 3.3.4, using the study by Hernandez et al. (2000) to assess the potential risks from inhalation exposures is a tenuous and probably extremely conservative approach—it tends to overestimate risk. Notwithstanding this limitation, it is the best available study from which the potential for serious adverse effects can be assessed. As in the human health risk assessment, a dose of 10^7 cfu/mouse is taken as the NOAEL and 10^8 cfu/mouse is taken as a frank effect level—a dose associated with 80% mortality.

As discussed in Section 4.1, adverse effects were not observed in mammals or birds after oral exposure to *B.t.k.*. Long-term doses up to 8400 mg/kg/day do not appear to cause adverse effects in mammals (McClintock et al. 1995b), and multiple oral doses up to 2857 mg formulation/kg bw are not associated with adverse effects in birds (Lattin et al. 1990a,b,d). For

this risk assessment, the dose of 8400 mg/kg/day is used as the NOAEL and is compared wth the exposure assessment developed for the small mammal (see Section 4.2.2.1).

4.3.2.2. Terrestrial Invertebrates – For terrestrial invertebrates, sufficient data are available to estimate dose-response relationships for sensitive species as well as relatively tolerant species. The data used in these analyses are summarized in Table 4-5. The sensitive species are all lepidoptera, and all of the studies used in the analysis involve feeding various lepidopteran larvae with vegetation treated with various *B.t.k.* formulations at rates that can be expressed in units of BIU/ha. Seven species of lepidoptera are included: two target species (the gypsy moth and cabage looper) and five non-target species (the Karner blue butterfly, two species of swallowtail butterfly, the promethea moth, and late instars of the cinnabar moth). The tolerant species used in the dose-response assessment involve feeding of early instar cinnabar moth larvae as well as direct spray of non-lepidopteran insects: green lacewing adults as well as larvae and direct spray of adult lady beetles. Details of these studies are presented in Section 4.1.2.3.

The analysis of these data is somewhat more elaborate than that in other sections of this risk assessment both because the data are sufficient for a more elaborate analysis and because the analysis is important. In plain language, the analysis derives dose-response relationships for both sensitive and insensitive species—i.e., estimates of mortality can be made for any application rate. Sensitive species have an LD_{50} value of about 21 BIU/ha and consist entirely of lepidoptera. The tolerant species have an LD_{50} of about 590 BIU/ha, which is approximately 28 times greater than the LD_{50} value for sensitive species. The details of these analyses are provided in the remainder of this section.

In Table 4-5, which summarizes the data used in the dose-response assessment for non-target insects, the first column specifies the common name of the test organism. This column is followed by the application rate in units of BIU/ha, the mortality rate (as a proportion of organisms) observed in control organisms not exposed to *B.t.k.*, and the mortality rate (again as a proportion) in treated organisms. The fifth column gives the mortality rate attributable to *B.t.k.* considering the control response. This rate is calculated using Abbott's formula:

$$\boldsymbol{P} = (\boldsymbol{P^*} - \boldsymbol{C}) / (1 - \boldsymbol{C})$$

where $\boldsymbol{P}$ is the proportion responding that is attributable to the agent, $\boldsymbol{P^*}$ is the observed proportion responding in the group exposed to the agent, and $\boldsymbol{C}$ is the proportion responding in the control group (Finney 1972, p. 125). This is a common method used to adjust mortality rates and assumes that the causes of mortality in the control group are independent of mortality attributable to the agent under study. As noted by Finney (1972), this is the standard approach for calculating the probability of combinations of independent events.

For statistical analysis, the probit model was used, which is similar to the approach taken in the analysis of the mortality data from Hernandez et al. (2000) in Section 3.3.4. Because different studies are combined, each with different control response rates, standard probit analysis was not used. Instead, the responses attributable to *B.t.k.* based on Abbott's formula were converted to probits using the inverse normal function in EXCEL:

$$\text{Probit} = 5 + \text{NORMINV}(\boldsymbol{P},0,1)$$

where 0 and 1 are the mean and standard deviation of the standard normal curve, and $\boldsymbol{P}$ is as defined above. The constant of 5 is the standard constant for converting normal equivalent

deviates to probits. Thus, a probit of 5 represents a response of 50%, a probit of 6 represents a response that is one standard deviation above 50% (i.e., a response of about 82%), a probit of 7 represents a response that is two standard deviations above 50% (i.e., a response of about 98%) and so on.

While it is beyond the scope of this risk assessment to discuss the probit transformation in detail, this transformation is simply a method to linearize the proportion responding under the assumption that the distribution of tolerances in a population (in this case the population of insects) has a log-normal distribution. Further details regarding the biological and statistical rationale for the probit transformation are provided in Finney (1972, p. 8 ff).

Using this transformation, the probit responses (independent variable) and $\log_{10}$ BIU/acre are used to estimate the linearized dose-response function:

$$\boldsymbol{Y} = \boldsymbol{a} + \boldsymbol{bx}$$

using standard linear regression where $\boldsymbol{Y}$ is the probit response, $\boldsymbol{x}$ is the $\log_{10}$ of the BIU/acre treatment, $\boldsymbol{b}$ is the slope of the dose-response curve, and $\boldsymbol{a}$ is the intercept.

The log-dose probit-response model provides a statistically significant fit to data for the sensitive ($p \approx 0.0004$, adjusted $r^2 = 0.79$) and the tolerant ($p \approx 0.00003$, adjusted $r^2 = 0.95$) species. In addition, the slopes of the dose-response curves are similar and not significantly different—i.e., 1.95 with a 95% confidence interval of about 1.2 to 2.7 for sensitive species and 2.6 with a 95% confidence interval of about 2.1 to 3.2 for tolerant species.

Consequently, the regression analysis was run a second time using a variable, $\boldsymbol{S}$, assigned a value of 1 for sensitive species and 0 for tolerant species in order to constrain the slopes of the two curves to be equal:

$$\boldsymbol{Y} = \boldsymbol{a} + \boldsymbol{bx} + \boldsymbol{cS}$$

where $\boldsymbol{c}$ is the coefficient for the sensitivity variable, $\boldsymbol{S}$, and the other terms are as defined above.

The data on both sensitive and tolerant species combined fits the following model:

$$Y = -1.48 + 2.34\,\boldsymbol{x} + 3.36\,\boldsymbol{S}$$

with a highly significant $\boldsymbol{p}$-value (8.4×10^{-11}) and an adjusted r^2 of about 0.95—i.e., the model explains 95% of the variability in the data ,and the probability that the association occurred by random chance is about 1 in 11 billion. It is worth noting that the p-value for the variable for sensitivity is about 2.8×10^{-11}, indicating a highly significant difference between the sensitive and tolerant species—i.e., the probability that the apparent difference occurred by chance is about 1 in 36 billion.

The above equation can be used to calculate the LD_{50} values for both tolerant and sensitive species in order to quantify relative potency, defined as the ratio of equitoxic doses. For sensitive species, this is done by setting $\boldsymbol{Y}$ equal to 5 and $\boldsymbol{S}$ equal to 1. With these substitutions, the value of $\boldsymbol{x}$, the log BIU/ha, is about 1.33, corresponding to an LD_{50} of 21 BIU/ha [$10^{1.33}$]. For tolerant species, the log of the LD_{50} is calculated by setting $\boldsymbol{Y}$ equal to 5 and $\boldsymbol{S}$ equal to 0 to yield a log BIU/ha of about 2.77, corresponding to an LD_{50} of about 590

BIU/ha [$10^{1.33}$]. Thus, the relative potency of *B.t.k.* to sensitive species is about 28, relative to tolerant species [590 BIU/ha ÷ 21 BIU/ha].

Figure 4-1 also contains data from the study in honey bees by Atkins (1991a) and data from Peacock et al. (1998) on a number of different non-target lepidoptera exposed to Foray 48B at 89 BIU/ha (Table 4-1 of this risk assessment) and Dipel 8AF at 99 BIU/ha (Table 4-2 of this risk assessment). In Peacock et al. (1998) study, several of the bioassays resulted in either 0% or 100% mortality. Neither of these values can be directly translated to probits. Thus, working probits of 3 were used for 0% mortality and working probits of 7 were used for 100% mortality, which reflect the approximate range of probit values from Peacock et al. (1998) in which partial mortality was observed. These values are used only to illustrate the data and were not used in any statistical analyses.

Figure 4-1 illustrates how the models fits the available data on sensitive and tolerant species. It is apparent from Figure 4-1 that the variability in sensitivity among the lepidopteran species reported by Peacock et al. (1998) is encompassed by the dose-response curves for sensitive and tolerant species derived from the data in Table 4-5, although the use of working probits for 0% and 100% mortality may obscure some of the more or less sensitive species. Given the available data, this apparent confusion cannot be avoided. As illustrated in Figure 4-2, the number of insensitive species (n=16) is somewhat greater than the number of sensitive species (n=10). Most species (n=28) appear to have intermediate sensitivity which is nearly uniformly distributed between that of sensitive and insensitive species. This figure is constructed by combining the data on both Foray 48B (Table 4-1 of this risk assessment) and Dipel 8AF (Table 4-2 of this risk assessment). Although the data on bees by Atkins (1991a) is also encompassed by the two dose-response curves, the slope of the dose-response relationship for bees appears to be more shallow than that of either dose-response curve.

In the context of this analysis, the designations of sensitive and tolerant species are not intended to imply absolute ranges on tolerance among all possible insects. Instead, the analysis simply indicates that some non-target 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. As illustrated in the data from Peacock et al. (1998), the range of sensitivities among various insect species appear to follow a continuum and it is possible that some species may be more or less sensitive to *B.t.k.* than indicated by the two dose-response curves illustrated in Figure 4-1.

4.3.3. Aquatic Organisms

4.3.3.1. Fish – With the exception of the recent publication by Meher et al. (2002), the detailed studies regarding the toxicity of *B.t.k.* and *B.t.k.* formulations are unpublished. These studies are summarized Appendix 5, which also summarizes data from secondary sources (Abbott Labs 1992; Mayer and Ellersieck 1986) and from the abstract by Martin et al. 1997. As discussed in Section 4.1.3.1, the study by Martin et al. (1997) is the only report of adverse effects on fish at concentrations that might result from the application of *B.t.k.* As further discussed in Section 4.1.3.1, this report is only in abstract form and a full publication of the study was not found in the literature. The results reported in the abstract are inconsistent with those reported in several more detailed full studies. Consequently, the information reported by Martin et al. (1997) is not used in the dose response assessment for fish. Similarly, the secondary sources (Abbott Labs 1992; Mayer and Ellersieck 1986) do not provide sufficient detail to evaluate the information reported. Given the availability of detailed primary studies on *B.t.k.* (Meher et al. 2002; Christensen 1990c,d,g,i), information from these secondary sources are not used in the dose-response assessment.

The study by Meher et al. (2002) involves a standard acute (96-hour) bioassay in mosquito fish at concentrations ranging from 200 to 1000 mg formulation/L. The study reports that the formulation contained 2.5×10^7 spores/mg. Assuming that the spores are viable, this range of concentrations corresponds to 5×10^9 to 2.5×10^{10} cfu/L. In this study, none of the fish died and there were no signs of sublethal toxicity—i.e., no effects on swimming behavior, reflexes, general appearance, and gill movement. Since *B.t.k.* will not persist in water (U.S. EPA 1998; Glare and O'Callaghan 2000), 1000 mg formulation/L or 2.5×10^{10} cfu/L is used as an NOEC to characterize potential effects in tolerant species of fish.

The series of studies by Christensen (1990c,d,g,i), however, were conducted over a longer period of exposure (about 30 days) and marginally significant mortality ($p=0.052$) was observed in rainbow trout at a nominal concentration of 2.87×10^7 cfu/L (Christensen 1990d). Christensen (1990d) specifies that the *B.t.k.* powder used in this bioassay contained 2.0×10^{10} cfu/g or 2.0×10^7 cfu/mg. Thus, the nominal concentration of 2.87×10^7 cfu/L corresponds to about 1.4 mg/L. While concentrations of *B.t.k.* in water will not remain constant for 30-days, the value of 1.4 mg/L or 2.87×10^7 cfu/L is used to characterize risk to sensitive species of fish.

As discussed further in the risk characterization (Section 4.4), the distinction between sensitive and tolerant species of fish has no impact on the risk assessment because the concentration of 2.87×10^7 cfu/L is far higher than any plausible concentrations of *B.t.k.* in water even over very brief periods of time. Consequently, there is no need to elaborate on the dose-response assessment for fish.

4.3.3.2. Invertebrates – As with terrestrial invertebrates, the toxicity data on aquatic invertebrates is much more diverse than the data on fish. As summarized in Appendix 6, laboratory toxicity bioassays are available in several different groups of aquatic invertebrates, and several field or field simulation studies are available on mixed populations of invertebrates. Comparisons among the different studies are confounded somewhat by the different units in which the results are reported —i.e., mg formulation, IU, or cfu per volume of water and application rates in units of BIU per area. Appendix 6 provides some estimated conversions for key studies.

The most sensitive species appears to be *Daphnia magna* with a 21-day EC_{50} for immobilization of 14 mg/L and a decrease in reproduction rates (number of young per surviving adult) at 5 mg/L using an unspecified Dipel formulation (Young 1990). Citing this study, U.S. EPA (1998) classifies *B.t.k.* as "moderately toxic" to daphnids. U.S. EPA (1998) does not cite the chronic study in daphnia by Christensen (1991d). In this study, adverse effects (mortality and decreased reproduction) were seen at a concentration of 5.9 mg/L or 6.24×10^8 cfu/L, consistent with the decreased reproduction reported by Young (1990) at 5 mg/L. The study by Christensen (1991d), however, provides a chronic daphnid NOEC of 0.45 mg/L or 6.24×10^8 cfu/L for both reproductive effects as well as mortality. This value is used to characterize risks in sensitive invertebrates. As noted in Appendix 6, the NOEC of 0.45 mg/L is somewhat below the estimated NOEC of 0.5 mg/L for effects on larvae of the blackfly (*Prosimulium fascum/mixtum*).

Some invertebrates, including copepods, caddisflies, and glass shrimp appear to be extremely tolerant to *B.t.k.* in laboratory bioassays. As noted in the risk characterization (Section 4.4), selection of a tolerant species has a limited impact on the risk assessment because relatively sensitive species do not appear to be at substantial risk. For this risk assessment, the NOEC of 36 mg/L is used to characterize risk for tolerant species of invertebrates. This value is taken from a series of 24-hour bioassays conducted by Kreutzweiser et al. (1992) in six species of

mayflies (Ephemeroptera), three species of stoneflies (Plecoptera), and three species of caddisflies (Tricoptera). At a concentration of 600 IU/ml, equivalent to a concentration of about 36 mg Dipel 8AF/L, no mortality was observed in four species of mayflies and three species of caddisflies. Mortality rates of 4% to 30% were noted in three species of stoneflies, two species of mayflies, and one species of caddisfly.

4.4. RISK CHARACTERIZATION

4.4.1. Overview.

An overview of the risk characterization for *B.t.k.* is presented in Table 4-6. The only organisms that are likely to be affected by *B.t.k.* or *B.t.k.* formulations are terrestrial insects. Separate dose-response curves can be generated for both sensitive and tolerant terrestrial insects. At the application rates used to the control of the gypsy moth, the expected mortality rates for sensitive terrestrial insects range from about 80% to 94%. All sensitive terrestrial insects are comprised of lepidoptera, including some species of butterflies, like the endangered Karner blue, and some swallowtail butterflies and promethea moths. In some cases, lepidopteran sensitivity to *B.t.k.* is highly dependent on developmental stage. This is particularly true for the cinnabar moth, with late instar larvae being as sensitive as target species to *B.t.k.* and early instar larvae being among the most tolerant lepidoptera. Given the mode of action of *B.t.k.*—i.e., it must be ingested in order to be highly toxic—effects on even the most sensitive species are anticipated only when species are in a sensitive larval stage at the time of or shortly after *B.t.k.* application. Much lower mortality rates (on the order of less than 1% to about 4%) are anticipated in tolerant species, including non-lepidopteran insects and certain lepidoptera at a particular stage of development. The risk characterization for terrestrial mammals is unambiguous: under foreseeable conditions of exposure, adverse effects are unlikely. 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 invertebrates other than insects appear to be of no 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 are plausible for some soil invertebrates —i.e., Collembola or earthworms.

4.4.2. Terrestrial Organisms.

4.4.2.1. Terrestrial Vertebrates – The risk characterization for terrestrial mammals is unambiguous: under any foreseeable conditions of exposure, adverse effects are unlikely. The potential for serious adverse effects is acknowledged, based on the Hernandez et al. (2000) study involving the intranasal instillation of *B.t.k.* to mice. The apparent NOAEL for adverse effects, however, is 10^7 cfu/mouse. The maximum concentrations of *B.t.k.* in ambient air range from 100 to 5000 cfu/m^3, based on monitoring data and the corresponding maximum dose of 0.168 cfu/mouse is based on the upper range of the concentration (5000 cfu/m^3) and the breathing rate of the mouse (0.0000336 m^3/day). The resulting hazard index of 2×10^{-8}—0.168 cfu/mouse ÷ 10^7 cfu/mouse rounded to 1 significant digit—is a factor of 50 million below the level of concern. Therefore, although the risk characterization acknowledges the possibility of serious adverse effects, the upper range of plausible levels of exposure are far below levels associated with serious adverse effects. For oral exposures, the hazard identification is essentially negative—i.e., there is no indication that oral exposure to *B.t.k.* at any concentration will cause adverse effects. For the purpose of quantitatively expressing risk, the dose of 8400 mg/kg/day is used as a working NOAEL, although it is possible that higher doses might also be classified as NOAELs. Based on a very conservative exposure assessment involving oral (vegetation and drinking water) as well as dermal (direct spray) scenarios, the hazard index is 0.02, a factor of 50 below the working NOAEL.

As noted in the risk characterization for human health effects (see Section 3.4.3), a recent study by Hernandez et al. (2000) reports a substantial increase in mortality in mice pre-treated with an influenza virus and then exposed to various doses of *B.t.k.* In this study, increased mortality was observed at a very low dose—i.e., 100 cfu/mouse —which is a factor of one-million below the lethal dose in non-viral treated mice of 1×10^8 cfu/mice. As discussed in Section 3.4.3, the significance of the Hernandez et al. (2000) study to potential human health effects is difficult to

assess. For wildlife, the estimated maximum exposure of 0.186 cfu/mouse is far below the 100 cfu/mouse exposure at which the increased mortality was observed. Nonetheless, the Hernandez et al. (2000) study does not identify a NOEC for mice pre-treated with influenza virus. Thus, as in the human health risk assessment, the potential for interactions between *B.t.k.* and populations infected with influenza virus cannot be well assessed at this time and is likely to be an area of further study in the coming years.

4.4.2.2. Terrestrial Invertebrates – Sufficient data are available to estimate dose-response relationships for both sensitive species as well as relatively tolerant species in units used to measure application rates—i.e., BIU/ha. As discussed in Section 4.3.2.2, risks for terrestrial insects can be expressed using a log-dose probit-response curve:

$$Y = -1.48 + 2.34\,\boldsymbol{x} + 3.36\,\boldsymbol{S}$$

where Y is the probit response, $\boldsymbol{x}$ is the common log of the application rate in BIU/ha, and $\boldsymbol{S}$ is equal to 1 for sensitive species and 0 for tolerant species. Substituting the application rates of 49 BIU/ha and 99 BIU/ha into the above equation, mortality rates in units of probits can be explicitly estimated for sensitive and tolerant organisms at both application rates. As summarized in Table 4-6, high mortality rates in sensitive species are likely—i.e., rates of about 80% to 94%. Mortality rates in tolerant organisms are estimated to be much lower, in the range of 0.6% to 3.6%. Given the experimental scatter (Figure 4-1), these rates should be regarded as approximate. While confidence intervals could be derived for the dose-response curves, they would have no impact on the risk characterization.

The identification of tolerant and sensitive organisms, however, is not always straightforward. As summarized in Table 4-5, target species like the gypsy moth and cabbage looper are clearly sensitive. In addition, some species of butterflies, including the endangered Karner blue and some swallowtail butterflies and promethea moths appear to be as sensitive as the target species to *B.t.k.* exposure. For some lepidoptera, sensitivity to *B.t.k.* depends primarily on developmental stage. This is particularly evident in the case of the cinnabar moth, with late instar larvae being as sensitive as target species to *B.t.k.* exposure and early instar larvae being among the most tolerant lepidoptera. All of the more sensitive organisms are lepidopteran larvae. Given the mode of action of *B.t.k.*—i.e., it must be ingested in order to be highly toxic—effects on even the most sensitive species are anticipated only when the species is in a sensitive larval stage at the time of *B.t.k.* application or shortly thereafter.

Tolerant species appear to be comprised of non-lepidopteran insects as well as certain larval stages of some lepidoptera. As noted above, early instar larvae of the cinnabar moth appear to among the most tolerant lepidoptera. Based on the study by Peacock et al. (1998), owlet moths and some looper butterflies also appear to be relatively tolerant to *B.t.k.* As illustrated in Figures 4-1 and 4-2, other lepidopteran species/instars display sensitivities that are intermediate between those of the most sensitive and most tolerant organisms, and the distribution of tolerances appears to be nearly uniform. As summarized in Appendix 3, the apparently wide variability of sensitivity among different lepidopteran species is supported by the recent field study of Rastall et al. (2003), who noted statistically significant decreases in three nontarget lepidopteran species but either no change or statistically significant increases in other nontarget lepidopteran species associated with the application of *B.t.k.*

Thus, the risk characterization for terrestrial insects is highly variable. Mortality rates are likely to be high among sensitive lepidopteran species after any *B.t.k.* application that is effective for controlling the gypsy moth or other target species, whereas mortality rates are not

likely to be detectable or biologically significant among non-lepidopteran insects or tolerant lepidoptera at certain stages of development. The response in other lepidopteran species will be intermediate between sensitive and tolerant species. As discussed in Section 4.1.2.3.2, an older oil-based formulation of *B.t.k.*, Dipel 4L, decreased populations of Collembola as well as earthworms. Dipel 4L is not used in USDA programs. Nonetheless, any oil-based formulation of *B.t.k.* (or any other pesticide) might be expected to cause adverse effects in some soil invertebrates.

As summarized in Table 4-5 and illustrated in Figure 4-1, the toxicity data on honeybees are encompassed by the dose-response curves for sensitive and tolerant insect species but the apparent slope of the mortality curve for honeybees is shallower than that for other insect species. This observation, however, is based on only a single study (Atkins 1991a) and should not be subject to over interpretation. Nonetheless, the data from Atkins (1991a) suggests that mortality rates in bees sprayed directly with *B.t.k.* at application rates used to control the gypsy moth could be approximately 20%. In practice, applications of *B.t.k.* to control the gypsy moth are not associated with substantial mortality in bees, which may be due to foliar interception of the applied *B.t.k.*

4.4.3. Aquatic Organisms.

The risk characterization for both fish and aquatic invertebrates is based on a maximum concentration of 0.24 mg formulation/L. As discussed in the exposure assessment (see Section 4.2.4), this concentration is calculated from an application rate of 2 lbs/acre or 224.2 mg/m^2 using a water depth of 1 m. In other words, 0.24 mg formulation/L would be the concentration in water immediately after direct spray over water. In most applications, actual concentrations in water would be much less, as suggested by the monitoring data of Valadares de Amorin et al. (2001). For both fish and invertebrates, this concentration is typically compared to longer-term toxicity values—i.e., 30 days for fish and 21 days for aquatic daphnids. Thus, the resulting hazard quotients are likely to overestimate risk substantially.

As summarized in Table 4-5, none of the hazard quotients exceed one—i.e., there is no indication that adverse effects are likely in either tolerant or sensitive species. For tolerant species the interpretation is unequivocal: hazard quotients are below a level of concern by factors of 5000 for fish and more than 140 for invertebrates. For sensitive species of fish, the hazard quotient of 0.2 is below the level of concern by a factor of 5. Given that the toxicity value is based on a 30-day NOEC and given that *B.t.k.* will not persist in water, there is no basis for concern in even sensitive species of fish. The hazard quotient of 0.5 for sensitive species of invertebrates may be viewed with marginal concern in that it suggests that effects could be seen in shallower bodies of water. Again, however, the toxicity value is based on a 21-day study and it is not likely that concentrations of *B.t.k.* would be maintained at levels close to 0.24 mg/L for this period of time.

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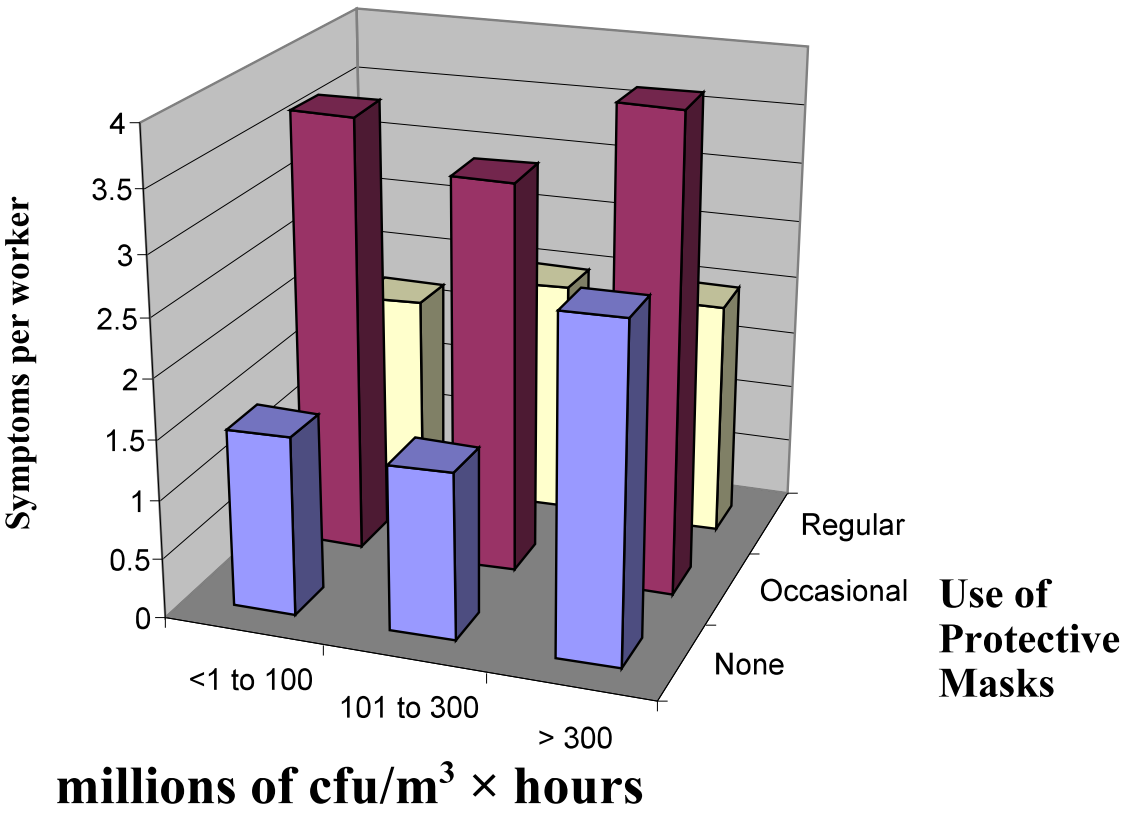

Figure 3-1: Number of symptoms per worker based on total exposure to *B.t.k.* (millions of cfu hours) and the use of protective masks (data from Cook 1994 as summarized in Table 3-6 of this risk assessment)

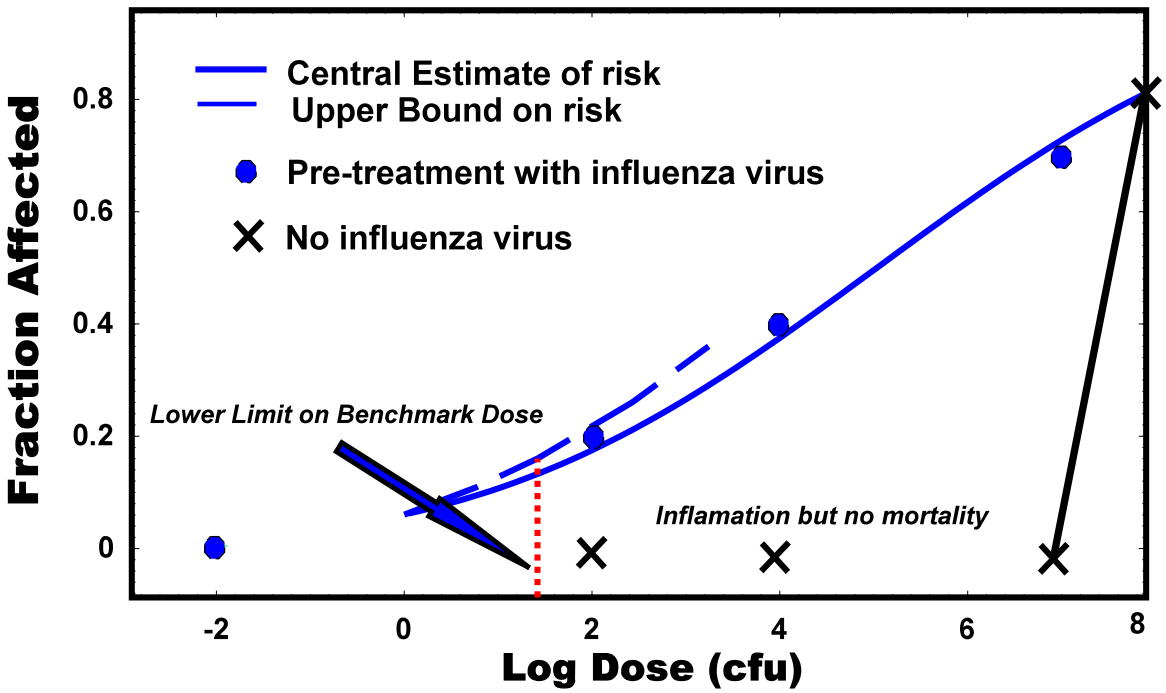

Figure 3-2: Dose-response relationships in mice after intranasal administration of *B.t.k.* with or without previous challenge with influenza virus at 4% of the LD_{50} (data from Hernandez et al. 1999 and 2000).

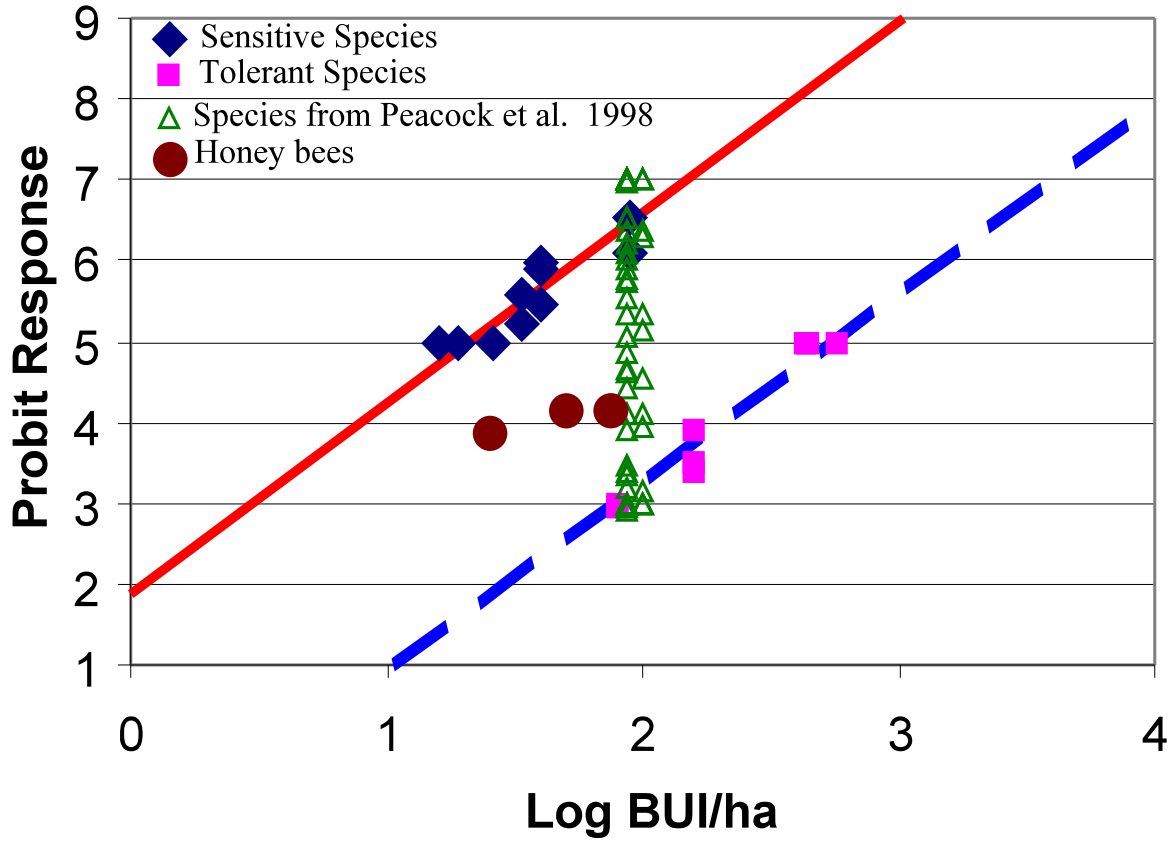

Figure 4-1: Dose-Response Assessment for non-target terrestrial invertebrates.

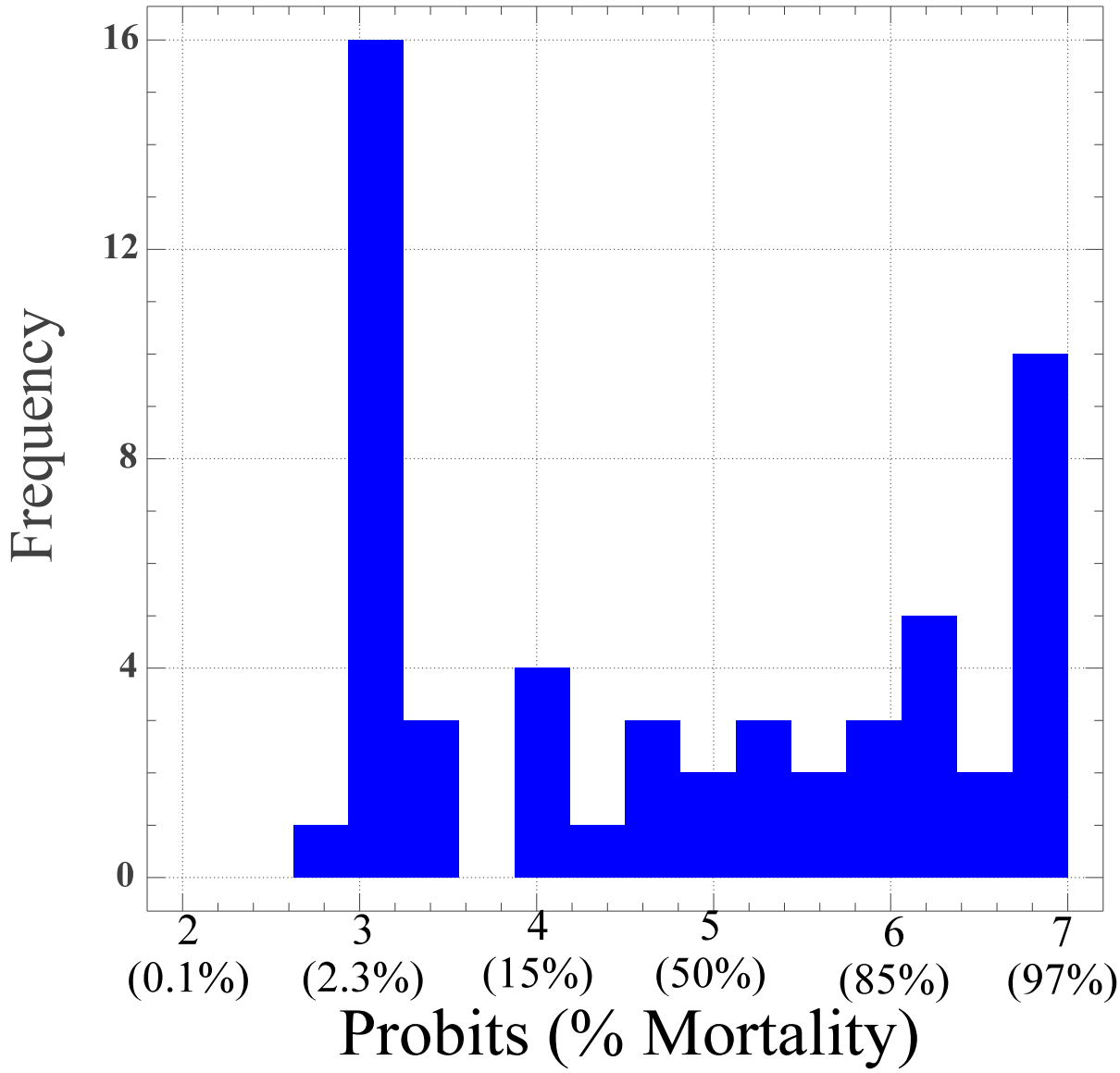

Figure 4-2: Distribution of sensitivity in various non-target lepidoptera (data from Peacock et al. 1998)

Table 2-1: Commercial formulations of *B.t.k.* that may be used in Forest Service Programs [1]

Formulation/ Producer	Type of formulation	% a.i. [2]	Potency	Application Rates [3]	Type application
Biobit HP/ Valent USA Corp	Wettable power	6.4	32,000 IU/mg 14.52 BIU/lb	0.5-2 lb/acre	Ground or aerial
DiPel DF/ Valent USA Corp	Dry flowable	10.3	32,000 IU/mg 14.5 BIU/lb	0.5-2 lb/acre	Ground only
DiPel ES/ Valent USA Corp	Emulsified suspension [6]	3.5	17,600 IU/mg 64 BIU/gallon	1-4 pints/acre	Ground only
DiPel Pro DF/ Valent USA Corp	Dry flowable	10.3	32,000 IU/mg 14.5 BIU/lb	1-4 lb/100 gallons	Ground only
DiPel 2X/ Valent USA Corp	Wettable powder	6.4	32,000 IU/mg 14.52 BIU/lb	0.5-2 lb/acre	Ground or aerial
Foray 48B/ Valent BioSciences	Flowable concentrate	2.1	10,600 UI/mg 48 BIU/gallon	1.3-6.7 pts/acre 8-40 BIU/acre	Ground or aerial
Foray 48F/ Valent BioSciences	Flowable concentrate	5.7	11,800 FTU/mg 48 BFTU/gallon	21-128 oz/acre 8-48 BFTU/acre	Ground or aerial
Foray 76B/ Valent BioSciences	Flowable concentrate	3.3	16,700 IU/mg 76 BIU/gallon	13.5-67.5 oz/acre 8-40 BIU/acre	Ground or aerial
[5] Thuricide 48LV/ Valent BioSciences	Aqueous concentrate	2.4	48 BIU/gallon	14-87 oz/acre 8-40 BIU/acre	Ground or aerial
[5] Thuricide 76LV/ Valent BioSciences	Aqueous concentrate	14.4	76 BIU/gallon	14-67 oz/acre 8-40 BIU/acre	Ground or aerial

[1] Source: Specimen labels from C&P Press, 2001.
[2] Includes *B.t.k.* solids, spores, and toxins. The remainder of the product formulation is classified as *inerts*. See text for discussion.
[3] All application rates expressed in amount (lb or oz) of formulation not amounts of active ingredient.
[4] Potency expressed as Forestry Toxic Units (FTU). Application rate corresponds to approximately 0.16 to 1 gallons/acre.
[5] Information based on Certis (2002) labels.
[6] Oil based formulation

TABLE 2-2: Use of *B.t.k.* from 1995 to 2001 for Suppression, Eradication, and Slow the Spread [1]

Year	Suppression	Eradication	Slow the Spread	Total
1995	271,961	332,276	32,528	636,765
1996	201,540	154,572	18,949	375,061
1997	46,703	200,720	18,744	266,167
1998	91,672	174,840	34,534	301,046
1999	153,198	164,856	7,252	325,306
2000	227,688	1,996	84,127	313,811
2001	273,384	1,440	62,398	337,222
2002	149,772	9,961	28,705	188,438
Total	1,415,918	1,040,661	287,237	2,743,816

[1] Source: ***GMDigest***, Morgantown, WV
(http://fhpr8.srs.fs.fed.us/wv/gmdigest/gmdigest.html)

Table 3-1: Epidemiology Studies on *B.t.k.* Formulations

Formulation, Location, Population, Exposure	Observations, Response	Reference(s)
Dipel, Oregon, USA, about 80,000 residents in spray area, 3 applications at 16 BIU/acre. About 180,000 residents in unsprayed area.	Surveillance program in four clinical laboratories for *B.t.k.* in clinical samples. Seven *B.t.k.* in clinical samples (other than incidental contamination) in sprayed area. None in unsprayed area. No significant adverse effects.	Elliott et al. 1988; Elliott 1986; Green et al. 1990
Foray 48B, British Columbia, Canada, residents in sprayed and unsprayed areas and workers, 20.2 BIU/acre.	Survey of 1,140 visits to family practice physicians and 3,500 hospital admissions. Analysis of Bacillus isolates. *B.t.k.* not implicated as disease agent. Cellular fatty acid profiles of *B.t.k.* cultures from humans as well as plants differed from *B.t.k.* in formulation. Some workers involved in ground applications evidenced nasal swabs positive for *B.t.k.* for up to 120 days after application. Respiratory and dermal irritation in workers.	Cook (1994); Noble et al. (1992)
Javelin (*B.t.k.* 17 BIU per lb), application rate not specified but probably in range of 2 BIU/acre to 25.5 BIU/acre, workers harvesting treated crops (groups of 20 to 48)	No signs of respiratory impairment or other adverse effects associated with exposure. A significant increase in skin-prick test responses to *B.t.k.* 1-4 months after exposure. Increase in IgE antibodies in highest exposure groups consistent with a potential for allergic sensitization.	Bernstein et al. 1999
Foray 48B, Auckland, New Zealand, 88,000 residents in sprayed area, 4.3 pints per acre (about 0.5375 gal./acre or 25.8 BIU/acre). Multiple applications in different areas.	Surveillance program of sentinel physicians. Self-reporting survey of adverse effects after exposure. Surveillance of births and incidence of meningococcal disease and reported infections. Self-reports of headache and respiratory irritation (sore throat). No effects demonstrated in review of sentinel physicians.	Aer'aqua Medicine Ltd. 2001
Foray 48B, British Columbia, Canada, 29 children in spray area and 29 children in unsprayed area, 3.4 pints/acre (about 0.425 gal./acre or 20.4 BIU/acre), 3 applications over 10 days.	No differences between the children (all with a history of asthma) in treated and untreated areas in terms of asthma symptoms or peak respiratory flow rates. No increase in symptoms of asthma in either group after spray.	Pearce et al. 2002
	Increase in incidence of *B.t.k.* HD-1 from nasal swabs after *B.t.k.* spray. Relatively few *B.t.k.* HD-1 identified in water (2.9%).	Valadares de Amorim et al. 2001
Foray 48B, Auckland, New Zealand, 292 individuals surveyed before and after spray, 4.3 pints per acre (about 0.5375 gal./acre or 25.8 BIU/acre). Three applications.	Self-reports before spray (n=292) and after spray (181 of 292 respondents). Increase in symptoms grouped as irritant, gastrointestinal, and neuropsychiatric effects that were significant at $p<0.05$ based on pair-wise comparisons.	Petrie et al. 2003

Table 3-2: Publically available information on inerts used in *B.t.k.* formulations.

Ingredient	Description
Benzoic acid/sodium benzoate [1]	CAS No. 65-85-0. GRAS compound and approved food additive. Functions in pH control and as an antimicrobial (Clydesdale 1997).
Hydrochloric acid [1]	CAS No. 7647-01-0. GRAS compound and approved food additive. Functions in pH control (Clydesdale 1997).
Methyl paraben [1,2] (methyl hydroxybenzoate)	CAS No. 7775-19-1. U.S. EPA List 3 Inert [3]. Uses: Pharmaceutical aid (antimicrobial preservative). Used in some suntan lotions, hand lotions, and bubble bath formulations. Occurs naturally in some berries and fruits (Burdock et al. 2002). There appears to be adequate data on this compound to remove it from List 3.
Phosphoric acid	CAS No.7664-38-2. GRAS compound and approved food additive. Functions in pH control, fermentation aid, fumigant, antimicrobial, and sweetener (Clydesdale 1997).
Polyacrylic acid (carbopol) [1]	CAS No.25987-55-7 (calcium polyacrylate). U.S. EPA List 3 Inert [3]. Toxicity data on this compound appears to be incomplete.
Potassium phosphate [2]	CAS No.7778-77-0. GRAS compound and approved food additive. Functions in pH control agent, nutrient supplement, stabilizer or thickener, malting or fermenting aid (Clydesdale 1997).
Potassium sorbate [1]	CAS No. 24634-61-5. GRAS compound and flavoring agent. Functions as antimicrobial agent, pH control agent, antioxidant, flavor Flavoring agent or adjuvant, nutrient supplement, or coloring adjunct (Clydesdale 1997).
Propylene glycol [1]	CAS No. 57-55-6. GRAS compound and food additive. Functions as solvent antimicrobial agent, anti-caking agent or free-flow agent, drying agent, flavoring agent or adjuvant, antioxidant, emulsifier, or formulation aid (NOS) (Clydesdale 1997).
Sodium hydroxide [2]	CAS No. 1310-73-2. GRAS compound and food additive. Functions as pH control agent, processing aid, fumigant, washing or surface removal agent, dough strengthener, flour treating agent, oxidizing or reducing agent, flavoring agent, coloring adjunct (Clydesdale 1997).
Sodium sulfite [2]	CAS No.7757-83-7. GRAS compound and food additive. Functions as dough strengthener, flour treating agent, oxidizing or reducing agent, color or coloring adjunct, ph control agent, antioxidant, formulation aid (NOS) (Clydesdale 1997).
Sorbitol [1]	CAS No.50-70-4. GRAS compound and food additive. Functions as stabilizer or thickener, nutritive sweetener, flavoring agent, drying agent, pH control agent, solvent, coloring adjunct, texturizer, nutrient supplement (Clydesdale 1997).
Sulfuric acid [2]	CAS No.7664-93-9. GRAS compound and food additive. Functions as pH control agent, formulation aid, flavoring agent, flavor enhancer, processing aid (Clydesdale 1997).

[1] Painted Apple Moth Community Coalition (CC-PAM), http://www.moth.co.nz/homepage.htm
[2] Swadener 1994
[3] The U.S. EPA inerts list is available at http://www.epa.gov/opprd001/inerts/

Table 3-3: Overview of exposure data for workers and members of the general public. [1]

Concentrations of *B.t.k.* in air [2]	Description	Reference
	WORKERS	
0.2 to 15.8 × 10^6 cfu/m^3	Highest exposures in ground spray workers. Lower range associated with support personnel – i.e., auditors, public relations personnel, and card handlers.	Cook 1994
400 to 11,000 cfu/m^3	No clear association between applicators (pilots) in aerial application and support personnel. Five of 15 workers, including one pilot, had no detected exposure.	Elliott et al. 1988, Elliott 1986
	GENERAL PUBLIC	
1000 and 1600 cfu/m^3	Personal air samples of four individuals. Exposure noted in two – a grocery store clerk and a service station attendant. Two individuals had no detectable exposures (a church custodian and a mail carrier).	Elliott et al. 1988, Elliott 1986
200 to 4,200 cfu/m^3	Twelve general air samples at various locations. No colonies in seven samples, some of which were in work area – i.e., helicopter loading area.	Elliott et al. 1988, Elliott 1986
739 cfu/m^3	The average in the spray zone during spraying.	Teschke et al. 2001
77 and 244 cfu/m^3	Average outdoor and indoor concentrations at 5 to 6 hour after spraying. Note: Indoor concentrations were higher.	Teschke et al. 2001
739-770 cfu/m^3	96% of samples positive for *B.t.k.* inside spray area during spray.	Valadares de Amorim et al. 2001
484-551 cfu/m^3	95% of samples positive for *B.t.k.* outside spray area during spray.	Valadares de Amorim et al. 2001

[1]See Table 3-1 for a description of the epidemiology studies.
[2]Excluding non-detects which are discussed in the description column.

Table 3-4: Post-spray symptoms reported by ground-spray workers and controls [1]

Symptom	Number (%)		*p*-value [2]
	Controls (n=29)	Workers (n=120)	
Dermal (dry or itchy skin, chapped lips)	3 (10%)	41 (34%)	0.007630
Eyes (redness, itch, burning, puffiness)	4 (13%)	24 (20%)	0.317398
Headache	3 (10%)	8 (7%)	0.858536
Throat (dry, sore)	2 (7%)	35 (29%)	0.007868
Runny nose or stuffiness	4 (13%)	32 (27%)	0.109883
Respiratory (cough, tightness)	1 (3%)	24 (20%)	0.021899
Digestive (nausea, diarrhea)	3 (10%)	8 (7%)	0.858536
Total (all symptoms combined)	11 (38%)	76(63%)	0.011638

[1] Data from Cook (1994), Table 3, p. 22.
[2] *p*-value calculated using Fischer-Exact Test [*p*-value = 0.05 ÷ 7 = 0.0071].

Table 3-5: Summary of the number of symptoms per worker in 120 ground-spray workers segregated by exposure groups and use of protective masks [1]

Exposure Group [2]	Mask Use [3]		
	Regular	Occasional	None
<1 to 100	1.7 [3]	3.7 [7]	1.5 [33]
101 to 300	2.0 [3]	3.3 [3]	1.4 [43]
> 300	2.0 [1]	4.0 [3]	2.8 [24]

[1] Data from Cook (1994), Table 3, p. 23.
[2] *B.t.k.* exposure in $cfu/m^3 \times 10^6 \times$ hours
[3] Number of symptoms per worker [number of workers per group]

Table 3-6: Self-reported symptoms in individuals before and after the aerial application of *B.t.k.* [1]

Health Problem	Baseline (n of 292)	After Spray (n of 181)	Reported p-value	Fisher Exact Test
Headache	133	93	0.06	0.127201
Back pain	105	57	0.06	0.863310
Coughing	85	60	0.1	0.204836
Cold, flu	84	54	0.6	0.441418
Sleep problems	78	66	0.03	0.016637
Neck pain	70	45	0.89	0.454930
Leg pain during physical activity	69	35	0.37	0.887366
Shoulder pain	59	43	0.26	0.211994
Arm pain	50	34	0.48	0.366523
Stomach discomfort	48	46	0.03	0.012472
Irritated throat	47	58	0.0001	0.000048
Itchy nose	47	42	0.04	0.036631
Migraine	37	27	0.18	0.287439
Dizziness	32	31	0.01	0.038634
Wheezing	29	24	0.11	0.167014
Diarrhoea	27	30	0.03	0.013527
Gas discomfort	25	30	0.02	0.006847
Chronic eye irritation	24	25	0.07	0.038379
Eczema	23	13	0.99	0.671774
Pain in ears	23	19	0.49	0.208708
Chest pain	21	16	0.49	0.315260
Extra heartbeats	20	19	0.05	0.110163
Constipation	18	12	0.32	0.491525
Difficulty concentration	15	23	0.001	0.003170
Blurred or double vision	15	18	0.2	0.036674

[1] The number of responders per effect is based on the percent responses and numbers of individuals reported in Petrie et al. 2003. The p-values in column 3 are those reported by Petrie et al. (2003). Fisher exact tests calculated on-line at http://www.matforsk.no/ola/fisher.htm. [*p*-value 0.05 ÷ 25 = 0.002]

Table 3-7: Exposure conversions for mice and humans with effects noted in mice after intranasal instillations.

cfu/mouse	Mouse cfu/m^3 × hour [(1)]	Equivalent cfu/person [(2)]	Equivalent human cfu/m^3 × hour [(3)]	Effect in Mice [(4)]
1e+02	7.14e+07	3.5e+05	1.4e+05	
1e+04	7.14e+09	3.5e+07	1.4e+07	inflamation, no mortality
1e+07	7.14e+12	3.5e+10	1.4e+10	
1e+08	7.14e+13	3.5e+11	1.4e+11	80% mortality

[(1)] Based on a breathing rate of 0.0014 L/hour for a 0.020 g mouse, derived from U.S. EPA (1988a), Recommendations for and Documentation of Values for Use in Risk Assessment, Table 1-3, p. 1-11: L/day = $1.99\ _{Bwkg}^{1.0496}$. Note that 0.0014 L/hour is equivalent to 0.0000014 m^3/hour [1 m^3 = 1000 L] or 0.0000336 m^3/day.
[(2)] cfu/mouse × 70 kg/0.02 kg.
[(3)] Based on a human breathing rate for moderate activity of 2.5 m^3/hour from U.S. EPA (1989d), Exposure Factors Handbook, Table 3-1, p. 3-4.
[(4)] From Hernandez et al. (1999, 2000), intranasal instillations in mice without exposure to influenza virus.

Table 3-8: Risk characterization for serious health effects from exposure to *B.t.k.*

Exposure	cfu/m^3	Duration (hours)	Cumulative Exposure (hours × cfu/m^3)	Hazard Index
General public, lower range	100	24	2,400	0.00000024
upper range	5,000	24	360,000	0.000036
Aerial Workers, lower range	400	8	3,200	0.00000032
higher range	11,000	8	88,000	0.000009
Ground Workers, lower range	200,000	8	1,600,000	0.00016
higher range	15,800,000	8	126,400,000	0.01264
extreme range			400,000,000	0.04
Human NOAEL	1.00e+10	hours × cfu/m^3		

Table 4-1: Mortality in species subject to foliage treated with Foray 48B at 89 BIU/ha (Peacock et al. 1998).

Family	Species	Instar [1]	Control		Foray 48B at 89 BIU/ha		*p*-value [3]
			No. Alive	No. Dead	No. Alive	No. Dead	
Papilionidae, Swallowtail Butterflies	*Papilio glaucus*	1-3	10	0	0	20	<0.00001
Nymphalidae, Danaid and Brown Butterflies	*Speyeria diana*	2-3	10	0	1	15	<0.00001
	Limenitis arthemis astyanax	n/n-1	10	0	0	20	<0.00001
	Astercampa clyton	4-5	21	1	1	40	<0.00001
Geometridae, Looper Butterflies	*Alsophila pometaria*	n	19	1	11	7	0.0164
	Phiglia titea	n/n-1	20	0	43	7	**0.1801**
	Euchlaena obtusaria	n-1	12	0	18	0	**1**
	Ennomos magnaria	1	22	1	0	66	<0.00001
	Ennomos magnaria	1	17	14	0	27	<0.00001
	Lambdina fervidaria	1	17	1	10	26	<0.00001
	Eutrapela clemataria	H [2]	20	0	4	31	<0.00001
	Prochoerodes transversata	2	19	1	28	13	0.0237
Lasiocampidae, Lappet Moths	*Malacosoma disstria*	2	23	4	4	26	<0.00001
	Malacosoma disstria	n	20	0	1	44	<0.00001
Saturniidae, Silk Moths	*Hemileuca maia*	H	47	0	5	53	<0.00001
	Hemileuca maia	1	70	1	48	312	<0.001
	Hemileuca maia	1	20	0	0	51	<0.00001
	Hemileuca maia	2	109	1	0	111	<0.00001
	Antheraea polyphemus	1	16	4	3	57	<0.00001
	Actias luna	1	26	14	0	96	<0.00001
Lymantriidae, Tussuck Moths	*Dasychira obliquata*	4	20	0	27	1	**0.9999**
Noctuidae, Owlet moths	*Amphipyra pyramidoides*	n-1	19	2	6	24	<0.00001
	Amphipyra pyramidoides	n-1	20	0	11	37	0.0001
	Xystopeplus rufago	1,2	28	0	12	21	<0.00001
	Psaphida rolandi	n-1	19	1	18	22	0.0001
	Psaphida resumens	1,2	20	0	9	41	<0.00001
	Egira alternans	1	20	5	22	27	0.0059
	Egira alternans	2-3	18	0	35	2	**1**
	Zale aeruginosa	H	12	0	19	11	0.0173
	Eupsilia vinulenta	n-1/n-2	20	0	19	1	**0.9999**
	Eupsilia vinulenta	n-1/n-2	20	0	43	1	**0.9999**
	Sericaglaea signata	4	18	0	48	0	**1**
	Metaxaglaea semitaria	n	20	0	51	1	**0.9999**
Noctuidae, Owlet moths *(continued)*	*Chaetaglaea sericea*	n-1	20	0	20	0	**1**
	Chaetaglaea sericea	n-1	19	0	48	1	**0.9999**
	Sunira bicolorago	n/n-1	20	0	45	3	**0.5498**
	Sunira bicolorago	n	20	0	29	0	**1**

Table 4-1: Mortality in species subject to foliage treated with Foray 48B at 89 BIU/ha (Peacock et al. 1998).

Family	Species	Instar [1]	Control		Foray 48B at 89 BIU/ha		*p*-value [3]
			No. Alive	No. Dead	No. Alive	No. Dead	
	Xylotype capax	n-1	19	1	48	0	**0.2941**
	Orthosia alurina	n-2	19	1	29	0	**0.9999**
	Orthosia alurina	n-1	18	0	30	7	**0.0823**
	Orthosia hibisci	n-1	20	0	39	0	**1**
	Abagrotis alternata	n/n-1	29	0	50	0	**1**
	Abagrotis alternata	n/–1	18	0	13	0	**1**

[1] n designates last instar
[2] H designate hatchling
[3] Fischer Exact test

Table 4-2: Mortality in species subject to foliage treated with Dipel 8AF at 99 BIU/ha (Peacock et al. 1998).

Family	Species	Instar [1]	Control		Dipel 8AF at 99 BIU/ha		*p*-value [3]	Comparison to Foray
			No. Alive	No. Dead	No. Alive	No. Dead		
Geometridae, Looper Butterflies	*Asterocampa clyton*	4,5	21	1	2	20	<0.00001	
	Alsophila pometaria	n	19	1	11	21	<0.00001	Match
	Ennomos magnaria	1	17	14	0	47	<0.00001	Match
Lasiocampidae, Lappet Moths	*Malacosoma disstria*	2	23	4	0	28	<0.00001	Match
Lymantriidae, Tussuck Moths	*Dasychira obliquata*	4	20	0	26	0	**1**	Match
Noctuidae, Owlet moths	*Catocala vidua*	1	17	2	0	31	<0.00001	
	Amphipyra pyramidoides	n-1	19	2	3	35	<0.00001	Match
	Lithophane grotei	n-1/n-2	20	0	22	28	<0.00001	
	Lithophane unimoda	n-1	19	1	38	9	**0.1423**	
	Eupsilia vinulenta	n-2	20	0	19	9	0.0063	No match, different instars
	Chaetaglaea sericea	n-1	20	0	30	0	**1**	Match
	Sunira biclorago	n/n–1	20	0	41	0	**1**	Match
	Orthosia alurina	n–2	19	1	14	4	**0.1698**	Match
	Abagrotis alternata	n/–1	18	0	31	1	**0.9999**	Match

[1] n designates last instar
[2] H designate hatchling
[3] Fischer Exact test

Table 4-3: Summary of exposures used in ecological risk assessment.

Organism	Exposure(s)	Section
Small mammal	Inhalation: 100 to 5000 cfu/m^3 or 0.00336 to 0.168 cfu/mouse Food/Water/Dermal: 184 mg/kg bw	4.2.2.1.
Terrestrial Invertebrates	20 to 40 BIU/acre [49 to 99 BIU/ha]	4.2.2.2.
Aquatic Species	0.24 mg formulation/L 7680 IU/L	4.2.4.

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

Organism	Toxicity Value(s)	Section
Small mammal	**Inhalation** 10^7 cfu/mouse – NOAEL 10^8 cfu/mouse – Frank Effect Level **Oral** 8400 mg/kg/day – NOAEL	4.3.2.1. and 3.3.4
Terrestrial Insects	Sensitive Species: 21 BIU/ha [≈ 8.4 BIU/acre] LD_{50} Tolerant Species: 590 BIU/ha [≈240 BIU/acre] LD_{50} *(see text for discussion dose-response curves)*	4.3.2.2.
Fish	Sensitive Species: 1.4 mg formulation/L or 1.51×10^7 cfu/L – LOEC Tolerant Species: 1000 mg formulation/L or 2.5×10^{10} cfu/L – NOEC	4.3.3.1.
Aquatic Invertebrates	Sensitive Species: 0.45 mg/L or 6.24×10^8 cfu/L – NOEC Tolerant Species: 36 mg/L – NOEC	4.3.3.2.

Table 4-5: Data used in dose-response assessment for non-target insects.

Common Name	Exposure (BIU/ha)	Control Response	Exposed Response	Mortality Attributable to *B.t.k.*	Reference
Sensitive Insects					
Gypsy moth 1st instar	33.5	0.2	0.67	0.5875	Herms et al. 1997
Gypsy moth 1st instar	90	0.2	0.95	0.9375	
Karner blue butterfly larvae	33.5	0	0.72	0.72	
Karner blue butterfly larvae	90	0	0.86	0.86	
Swallowtail butterfly larvae	40	0.67	0.94	0.8182	Johnson et al. 1995
Swallowtail butterfly larvae	40	0.58	0.93	0.8333	
Promethea moth larvae	40	0.66	0.89	0.6765	
Cabbage looper larvae	16	0	0.5	0.5	James et al. 1993
Cinnabar moth, 4th instar	26	0	0.5	0.5	
Cinnabar moth, 5tht instar	19	0	0.5	0.5	
Tolerant Insects					
Cinnabar moth, 1st instar	427	0	0.5	0.5	James et al. 1993
Cinnabar moth, 2nd instar	437	0	0.5	0.5	
Cinnabar moth, 3rd instar	575	0	0.5	0.5	
Green lacewing, larvae	79	0.116	0.135	0.0215	Haverty 1982 [a]
Green lacewing, adult	79	0.037	0.056	0.0197	
Green lacewing, larvae	158	0.116	0.175	0.0667	
Green lacewing, adult	158	0.037	0.088	0.0530	
Lady beetle, adult	158	0.335	0.424	0.1338	
Other Insects [b]					
Honey bee, adult worker	25	0	0.127	0.127	Atkins 1991a [a]
	50	0	0.192	0.192	
	75	0	0.191	0.191	

[a] These studies involved direct spray of adults or larvae as specified in column 1. All other studies involved consumption of contaminated vegetation by larvae.
[b] Not used quantitatively in dose-response assessment. See text for discussion.

Table 4-6: Risk characterization for ecological risk assessment of *B.t.k.*

Species	Scenario or Group	Exposure	Toxicity Value	Risk Characterization [1]
Small Mammal	Inhalation	0.168 cfu	10^7 cfu	HQ = 2×10^{-8}
	Oral/Dermal	184 mg/kg	8400 mg/kg	HQ = 0.02
Terrestrial Insects	Sensitive Species	49 to 99 BIU/ha	Dose-response curve [2]	80% to 94% [Probit 5.84 to 6.55]
	Tolerant Species			0.6% to 3.6% [Probit 2.47 to 3.19]
Other terrestrial invertebrates	All	No effects anticipated from *B.t.k.* Oil based formulations may cause adverse effects in some soil invertebrates.		
Fish	Sensitive Species	0.24 mg/L	1.4 mg/L	HQ = 0.2
	Tolerant Species		1000 mg/L	HQ = 0.0002
Aquatic Invertebrates	Sensitive Species	0.24 mg/L	0.45 mg/L	HQ = 0.5
	Tolerant Species		36 mg/L	HQ = 0.007

[1] For all groups except terrestrial invertebrates, the risk characterization is given as the hazard quotient (HQ), the exposure divided by the toxicity value.
[2] Estimated mortality based on dose response equation: $Y = -1.48 + 2.34\,\boldsymbol{x} + 3.36\,\boldsymbol{S}$. In this equation, Y is the probit response, $\boldsymbol{x}$ is the common log of the application rate in BIU/ha, and $\boldsymbol{S}$ is equal to 1 for sensitive species and 0 for tolerant species. See text for discussion.

APPENDICES

Appendix 1: Toxicity in Mammals
Appendix 2: Toxicity in Birds
Appendix 3: Toxicity in Non-target Lepidoptera
Appendix 4: Toxicity in Non-Target Terrestrial Insects Other Than Lepidoptera
Appendix 5: Toxicity of *B.t.k.* and *B.t.k.* Formulations to Fish
Appendix 6: Toxicity of *B.t.k.* and *B.t.k.* Formulations to Aquatic Invertebrates

Appendix 1: Toxicity in Mammals			
Product	Species/Exposure	Observations	Reference
ORAL			
DiPel "technical material"	Rat/Sprague-Dawley, 21/male 21/female, 10^8 cfu, gavage	No mortality and no signs of toxicity. Total clearance estimated at 47 days based on fecal excretion. Some samples from tissues (kidney and spleen) contained *B.t.k.* but this was seldom demonstrated on duplicate plates. This was also seen in some control animals and attributed to contamination of plates.	David 1990b
DiPel Technical Powder	Rat/Sprague-Dawley, 4/male 5/female, 5050 mg/kg gavage	Mortality in one male rat on Day 1, probably due to aspiration of material during dosing. No treatment related signs of toxicity.	Bassett and Watson 1999a
Dipel ES	Rat/Sprague-Dawley, 5/male 5/female, 5050 mg/kg gavage	No mortality, no gross pathology, and no clinical signs of toxicity.	Kuhn 1998b
Foray 48B	Rat/Sprague-Dawley, 5/male 5/female, 5000 mg/kg gavage	No mortality; no clinical signs; no abnormalities at necropsy. [Identical data cited in summary by Berg et al. 1991.]	Cuthbert and Jackson 1991
Foray 76B	Rat/HSD, 5/male 5/female, 5050 mg/kg gavage	No mortality; all rats appeared normal for the duration of the study; gross necropsy revealed no abnormalities in any of the rats	Kuhn 1991
Foray 48B	Rat/Wistar 14/male 14/female, 1 mL/rat	No mortality; there was no treatment related pathology; after 4 days, *B.t.k.* was isolated from the lungs and spleen in one rat, which indicates a technical error at dosing; two other rats also showed the microorganism in the lungs after 15 and 22 days, respectively; the microbial count in feces decreased rapidly during the first 3 days after exposure.	Harde 1990a
B.t.k. (NOS) from Novo Nordisk	Rats, SPF Wistar, 4M/4/F, 1 mL dose (cfu counts in dose illegible on fiche). Gavage	No mortality or signs of toxicity. No *B.t.k.* found in blood. *B.t.k.* in feces and organs dropped by a factor of 100 in 24 hours.	Harde 1990a
sB.t.k. powder	Rats, Wistar, 10^8 cfu per rat, gavage. Groups of 3-4 rats per sex	No effect on mortality, organ weights, gross pathology, and clinical signs. *B.t.k.* not found in blood of any animal. *B.t.k.* decreased by factor of about 100 per day. No indication of infectivity based on microbial counts in kidney, liver, spleen, lymph nodes, lungs, brain, blood and feces.	Harde 1990b
B.t.k.	Rats, HA albino. 20M/20F, 7.5×10^7, 1×10^6, 1.25×10^6 spores/rat, single oral dose (presumably gavage)	No signs of toxicity over 21-day observation period based on mortality, body and organ weights, clinical biochemistry and hematology, and reflexes.	Meher et al. 2002
Note on Meher et al. 2002: *B.t.k.* characterized as a wettable powder formulation produced in India.			

Appendix 1: Toxicity in Mammals			
Product	Species/Exposure	Observations	Reference
DERMAL			
Dipel ES	Rabbits, 5/male 5/female, 5050 mg/kg, intact skin	No mortality. Decreased body weight in 6 animals. Signs of dermal irritation included erythema, edema, and desquamation.	Kuhn 1998b
Dipel ES	Rabbits, 3/male 3/female, 0.5 mL, intact skin, covered with patch. Removed after 6 hours.	Very slight erythema a 1 and 24 hours.	Kuhn 1999a
NOTE on Kuhn 1998b and Kuhn 1999a: Study titles on title page indicate that the studies were done on rats. This is clearly an error. The studies were conducted on New Zealand White rabbits.			
B.t.k. formul-ation	Rabbits, albino. 6M/6F, 2.5×10^7 spores in 1 mL on shaved and abraded skin	"Low-grade" reddening of skin which reversed after 72 hours. No signs of toxicity over 21-day observation period.	Meher et al. 2002
B.t.k. formul-ation	Rabbits, albino. 6M/6F, 5×10^7 spores in 0.5 mL on shaved and abraded skin. Treated area covered.	"Low-grade" reddening of skin which reversed after 72 hours.	Meher et al. 2002
DiPel Technical Powder	Rabbits, 6/female, 0.5 g on abraded skin	Well-defined erythema at 30 minutes to 24 hours in 3 rabbits, which reduced during the 14-day period. On rabbit with initial slight erythema from 30 minutes had well-defined erythema by Day 14.	Bassett and Watson 1999b
Foray 48B	Rabbit/Mol: Russian, 6/female, 0.5 mL, 4 hours	Very slight erythema in one rabbit	Jacobsen 1993
Foray 48B	Rabbit, 10^{10} cfu/rabbit	Mild irritation which cleared after 4 days.	Berg et al. 1991
Foray 76B	Rabbit/New Zealand White, 5/male 5/female, 2.0 g ($1x10^{10}$ units/rabbit), 24 hours	No systemic effects; only mild skin reactions that cleared within 2 days after exposure. Behavior and appearance of all rabbits were normal throughout the study; agent was classified as "mild irritant"	Kiehr 1991a
OCULAR			
Dipel ES	Rabbits, 3M/3F, 0.1 mL formulation in right eye for 1 minute and then washed.	At 1 hour post-exposure, redness in conjunctiva of 2 rabbits. Normal after 24 hours. No other effects on conjunctiva, iris, or cornea.	Kuhn 1999b
Foray 48B (Batch BBN 6056)	Rabbit/New Zealand White, 6/male, 0.1 mL	Conjunctival reactions in the form of redness and discharge that cleared within 7 days after application	Berg 1991a

Appendix 1: Toxicity in Mammals			
Product	Species/Exposure	Observations	Reference
Foray 48B (Batch BBN 6057)	Rabbit/New Zealand White, 6/male, 0.1 mL	At day 7 mild redness was seen in 3/6 rabbits accompanied by small amounts of discharge in one of them; at day 8 mild redness was still seen in 1 rabbit and small of amounts of discharge were seen in another; lesions were temporary and cleared within 9 days after application.	Berg 1991b
Foray 48B (Batch BBN 6057)	Rabbit/New Zealand White, 6/male, 0.1 mL	Substantial conjunctival reactions; lesions were of temporary nature and cleared within 10 days after application	Berg and Kiehr 1991
B.t.k. formul-ation	Rabbits, albino. 3M/3F, 2.5×10^6 spores in 0.1 mL into one eye.	No signs of irritation or other effects over 14-day observation period. At 14 days but not 20 day, *B.t.k.* could be detected in cultures from the treated eye.	Meher et al. 2002
INHALATION			
B.t.k. (Biobit concent-rate)	Rats, Sprague-Dawley: 14M/14F per dose. 0.47 and 2.17 mg/L, 4 hours, nose only.	No mortality. Respiratory depression during exposure. Transient body weight loss. Dose related increase in mottled lungs. Poorly eliminated from lungs over 28 days – i.e., very little change at low dose and decrease by a factor of about 10 at high dose (Appendix 3 of study).	Oshodi and Mac-naughtan 1990a
Note: Oshodi and Macnaughtan 1990c has different MRID number but appears to be identical to Oshodi and Macnaughtan 1990a. Probably two different submissions.			
Dipel ES	Rats, Sprague-Dawley: 5M/5F. 2.95 mg/L for 4 hours.	No mortality or clinical signs of toxicity. Gross necropsy noted discolored lungs in one male and two females.	Leeper 1999a
Dipel Technical Powder	Rat/Sprague-Dawley, 5/male 5/female, 5.95 mg/L for 4 hours. Whole body.	No mortality. Decrease in activity and piloerection on Day 1 only. No signs of toxicity over 14-day observation period.	Leeper 1999b
Foray 76B	Mice (M/F): aerosol whole body exposure, 4 hours, 3.22 mg/L. ($3.13x10^9$ cfu/L)	Decreased activity, alopecia, piloerection, polyuria. Alopecia at necropsy was considered unusual and possibly related to exposure; no rats died during the study; during exposure period the rats were heavily coated with the thick test material.	Holbert 1991
Foray 48B	Rat/Sprague-Dawley, 14/male 14/female, 0.47 mg/L for 4 hours	Respiratory depression during exposure; wet and unkempt appearance after exposure; gross pathology included mottled lungs (sometimes dark) in a majority of rats; histopathology revealed alveolitis, interstitial pneumonitis, perivascular eosinophils and focal intra-alveolar hemorrhage; minimal bronchiolitis was observed in a few animals.	Oshodi and Mac-naughtan 1990b
Foray 48B	Rat/Sprague-Dawley, 5/male 5/female, 6.81 mg/L for 4 hours, nose only	There was no mortality; necropsy revealed no observable abnormalities; all values for lung:body weight ratio were within normal limits	McDon-ald and Scott 1991

Appendix 1: Toxicity in Mammals			
Product	Species/Exposure	Observations	Reference
INTRATRACHEAL			
Dipel technical powder, 2.01×10^{10} spores/g	Rat/Sprague-Dawley, 0.06 mL of 9×10^{9} or 1.55×10^{10} cfu/mL to groups of 9M/9F and 24M/24F, respectively.	Respiratory distress, lethargy, hunched body position, and ruffled coat on Day 1. 10/33 males and 15/33 females died on Day 2. Sporadic deaths thereafter. *B.t.k.* found in spleen, liver, lymph nodes and kidney. On necropsy, severe pulmonary hemorrhaging and edema. Clearance time in surviving animals estimated at 235 days.	David 1990c
PARENTERAL			
Foray 48B	Rat/Wistar, 5/Male, i.v., 1 mL ($3x10^{9}$ cfu/g) [vehicle=0.9% sterile NaCl]	Four of five rats died within 23 hours. Edema and hemorrhages were seen in the pyloric part of the stomach in all rats; two rats had enlarged spleens; the rat that was killed had a necrotic tail and extensive oedema and hemorrhages on the hindquarters stretching down on the hind legs.	Berg 1990
Foray 48B	Rat/Wistar, 16/Male, 16/Female, iv, 1 mL ($4x10^{8}$ cfu/g) [vehicle=0.9% sterile NaCl]	No mortality; transient decreased motor activity and cyanotic appearance 30 minutes after exposure; enlarged spleen in 2 rats; treatment-related unspecific reactive hepatitis; A higher incidence of histopathological findings in the liver and the reticuloendothelial system was found in the treated group compared to the controls. These were attributed to a background viral infection suggesting that the treatment with high levels of *B.t.k.* aggravated a preexisting disease. Over 167 days, a complete elimination of the test organism from all tissues except the spleen, which on average contained $3x10^{2}$ *B.t.k.*/g at the end of the study.	Berg 1990
B.t. strain SA-3	Mice, 3M/3F per dose, i.p. injections of 10^{6}, 10^{7}, and 10^{8} cfu/mouse.	No mortality or clinical signs of toxicity.	Schindler 1990a
B.t. strain SA-3	Mice, 5M/5F per dose, i.p. injections of 10^{6}, 10^{7}, and 10^{8} cfu/mouse.	No mortality or clinical signs of toxicity. Enlarged spleen and kidney in one female at low dose not attributed to treatment.	Schindler 1990b
B.t. strain SA-10	Mice, 5M/5F per dose, i.p. injections of 10^{6}, 10^{7}, and 10^{8} cfu/mouse.	No mortality or clinical signs of toxicity. Enlarged spleen in 1/5, 1/5, and 3/5 animals in the low, mid, and high dose groups. Variable changes in kidney weight. These effects were not attributed to treatment.	Schindler 1990c
B.t. strain SA-12	Mice, 5M/5F per dose, i.p. injections of 10^{6}, 10^{7}, and 10^{8} cfu/mouse.	4/5 males and 3/5 females died 1 to 3 days after injections at the highest dose. Signs of toxicity observed in surviving animals – including hypoactivity, enlarged spleens, and effects on the kidneys.	Schindler 1990d
NOTE: SA-12 is 3a3b, *B.t.k.* (Chen and Macuga 1990o,p,q)			

Appendix 1: Toxicity in Mammals			
Product	Species/Exposure	Observations	Reference
B.t.k. CGA-237218	Mice (5M/5F): 10^6, 10^7, 10^8 cfu/mouse. Five different production batches.	No mortality in any batch at lowest dose. At mid-dose, no mortality in 3 batches and 10% and 40% mortality in two batches. At highest dose, 50% to 100% mortality.	Vlachos 1991
NOTE: CGA-237218 is not identified in Vlachos (1991) but is clearly identified as *B.t.k.* in Christensen (1991c).			
FIELD STUDIES			
B.t.k. (Dipel 8L and red dye)	Masked shrew (*Sorex cinereus*) exposed to aerial application of 1.8 L/ha (30 BIU/ha or ca. 12 BIU/acre) Dipel 8L on a 22-year-old jack pine plantation in northern Ontario between May and July 1989.	Treatment had no effect on the total abundance of *S. cinereus*; however, the investigators observed treatment-related effects on the abundance and diet of certain sex and age groups: there were fewer adult males and more juveniles in the treated areas, compared with the control areas. In addition, adult males in the treated area at the same proportion of lepidopteran larvae as in the control area, while females and juveniles shifted their diet form lepidopteran larvae to alternate prey, which may have been due to the significant reduction in lepidopteran larvae as a result of treatment.	Belloq et al. 1992
B.t.k. (Thuricide 48 LV)	Populations of small rodents and shrews. 20 BIU/ha (ca. 8 BIU/acre)	No detectable impact on populations.	Innes and Bendell 1989
Omitted some studies in which the *B.t.* strain was not identified (Robbins 1991a,b). Omitted studies of Abbott ABT-6305 in this and other tables. Abbott ABT-6305 is *B.t. aizawai* (www.epa.gov/pesticides/foia/reviews/006403.htm).			

Appendix 2: Toxicity in Birds			
Product	Species/Exposure	Observations	Reference
ORAL			
B.t. EG2348	Bobwhite Quail, 3333mg/kg gavage	No mortality or signs of toxicity/pathogenicity.	Beavers et al. 1988a
B.t. EG2348	Mallard Duck, 3333mg/kg gavage	No mortality or signs of toxicity/pathogenicity.	Beavers et al. 1988a
Biobit WP	Mallard Duck, 2500 mg/kg or about 5.7×10^{11} cfu/kg for 5 days by gavage.	No signs of toxicity or pathogenicity.	Lattin et al. 1990c
Biobit WP	Mallard duck, 2500 mg/kg or about 2×10^{11} spores/kg by gavage for 5-days	No signs of toxicity or pathogenicity.	Lattin et al. 1990g
Dipel *B.t.k.*	Bobwhite quail, 2857 mg/kg or about 5.7×10^{10} spores/kg for 5 days by gavage.	No signs of toxicity or pathogenicity.	Lattin et al. 1990a
Dipel *B.t.k.*	Mallard Duck, 2857 mg/kg or about 5.7×10^{10} spores/kg for 5 days by gavage.	No signs of toxicity or pathogenicity.	Lattin et al. 1990b

Appendix 2: Toxicity in Birds			
Product	Species/Exposure	Observations	Reference
Dipel Technical Material	Bobwhite quail, 2857 mg/kg or about 5.7×10^{10} spores/kg for 5 days by gavage.	No signs of toxicity or pathogenicity.	Lattin et al. 1990d
Biobit *B.t.k.*	Bobwhite quail, 2500 mg/kg or about 2×10^{11} spores/kg for 5 days by gavage.	No signs of toxicity or pathogenicity.	Lattin et al. 1990e
Biobit *B.t.k.*	Mallard duck, 2500 mg/kg or about 2×10^{11} spores/kg for 5 days by gavage.	No signs of toxicity or pathogenicity.	Lattin et al. 1990f
B.t. Abbott ABG-6305	Bobwhite quail, 1714 mg/kg or about 3.4×10^{11} cfu/kg for 5 days by gavage.	No signs of toxicity or pathogenicity.	Lattin et al. 1990f
B.t. Abbott ABG-6305	Mallard duck, 1714 mg/kg or about 3.4×10^{11} cfu/kg for 5 days by gavage.	No signs of toxicity or pathogenicity.	Beavers 1991b
Omitted studies by Beavers and Smith 1990a,b on Delta BT. Cannot identify as *B.t.k.* Omitted Beavers 1991a,b on *B.t.* Abbott ABG-6305. This is *B.t.a.*			
FIELD STUDIES			
B.t.k. Thuricide 23LV with Rhoplex sticker	Black-throated blue warblers (*Dendroica caerulescens*), aerial application of 3.5 L/ha to four 30-hectare forested plots of White Mtn. National Forest, NH consisting of second-growth northern hardwoods (predominantly sugar maple, american beech, and yellow birch). The study was conducted between 1982 and 1985.	In 1983, caterpillar biomass was significantly different throughout the breeding season in ***one*** sprayed plot, compared with two unsprayed plots. Other adverse effects on the reduced caterpillar plot included significantly fewer nesting attempts and significantly fewer caterpillars in the diets of nestlings. No adverse effects were observed on clutch size, hatching success, or the number of fledglings per nest in the reduced food site, compared with controls. Spraying had no detectable effects on caterpillar biomass in 1984 or 1985 because the natural abundance of caterpillars was already low. Investigators conclude that *neotropical migrant bird species are probably limited periodically by food when breeding in north-temperate habitats*.	Rodenhouse and Holmes 1992
B.t.k. (NOS)	Hooded warbler (*Wilsonia citrina*) on two treatment plots in the Arkansas Ozards following two applications of *B.t.* in 1994	*B.t.k.* application appeared to have only minimal adverse effects on reproduction, in as much as the decreased numbers of lepidotperan larvae appeared to have a negative effect on nestling masses early in the season and appeared to alter feeding rates only in small clutches.	Nagy and Smith 1997

Appendix 2: Toxicity in Birds			
Product	Species/Exposure	Observations	Reference
B.t.k. (NOS)	Chestnut-backed and black-capped chickadees (*Parus rufescens*, and *P. atricapillus*), application of unspecified product at 60 BIU/ha in Portland, OR area and surrounding counties.	No effects on growth rate of fledgling success in 1st year. Reduced fledgling success 2nd year due to unexplained nest abandonment on 3 treatment plots (also 1 nest on control plot). Significantly smaller proportion of caterpillars brought as food on treatment sites both years, but provisioning rate no different.	Gaddis 1987; Gaddis and Corkran 1986 as cited in USDA/FS 1995
B.t.k. , Thuricide 48 LV	20 BIU/ha for control of jack pine budworm. Aerial and hand spray.	Assay of secondary effects on chicks of spruce grouse (*Dendragapus canadensis*). Chicks (dependent on larvae for first two weeks) were allowed to graze freely on either treated or untreated plots. About a 50% decrease in lepidopteran larvae on treated plots. Slower growth rate for chicks on treated plots. Based on linear slopes (Figure 2), growth rate was decrease by about 33%. Attributed to change in larvae availability on treated plots.	Norton et al. 2001
B.t.k. , Foray 48B	Foray 48B applied at 50 BIU/ha. Three applications.	Assayed song bird populations on treated and untreated plots before and after applications in the same year as well as assay approximately one year after applications. In general, no adverse effects on songbird populations in terms of species richness and relative abundance of song birds despite a decrease in caterpillar populations. In one species of 42 species surveyed, the spotted towhee (*Pipilo maculatus*), a statistically significant decrease in abundance was noted in the spray year but not one year following the spray.	Sopuck et al. 2002

Appendix 3: Toxicity in Non-target Lepidoptera			
Product	Species/Exposure	Observations	Reference
B.t.k. (Thuricide 16B; Dipel WP, with or without chitinase)	Spruce budworm (*Choristoneura fumiferana*) exposed to applications of 2 or 4 lbs/acre in Algonquin Park, Ontario and Spruce Woods Manitoba (Spruce-Fir forests).	No differences in treated or control plots regarding the number of hand-picked larvae from aspen, alder, and maple.	Buckner et al. 1974
B.t.k. (NOS)	32 Species of Lepidoptera on tobacco brush (*Ceanothus velutinusI*) treated with 20 BIU/ha (product not specified) in program to control spruce budworm (*Choristoneura occidentalis*) in Estacada, Clackamas County, OR	Number of larvae on shrubs in treated site decreased 80% between pre- and post-treatment surveys, compared with controls site where the number of larvae increased 6% in the same time period, 2 weeks after treatment; there were no differences between spray and control sites 2 months after treatment.	Miller 1990a
B.t.k. (NOS)	35 Species belonging to 10 families in the guild of nontarget leaf-feeding Lepidoptera (caterpillars) on Garry oak (*Quercus garryana*) monitored in the field from 1986 to 1988 in Elmira, Lane County, OR after three aerial (via helicopter) applications of 16 BIU/2.8 L water/0.4 ha *B.t.k.* Target species was the gypsy moth.	Target species was significantly reduced in treated plots during all 3 years of the study; species richness was reduced in the treated plots during all 3 years of the study; and the total number of individual non-target Lepidoptera was significantly reduced in treated plots in years 1 and 2 but not in year 3.	Miller 1990b
B.t.k. Thuricide 23LV with Rhoplex sticker	Forest Lepidoptera, aerial application of 3.5 L/ha to four 30-hectare forested plots of White Mtn. National Forest, NH consisting of second-growth northern hardwoods (predominantly sugar maple, American beech, and yellow birch). The study was conducted between 1982 and 1985.	Significant decrease in caterpillar biomass in treated plots, compared with untreated plots, in 1983; no significant decreases in caterpillar biomass between treated and untreated plots in 1984 or 1985 because natural abundance was already low.	Rodenhouse and Holmes 1992
B.t.k. (NOS)	Non-target moths in Asian gypsy moth eradication program in Pierce and King Counties, WA exposed to 60 BIU/ha (24 BIU/acre).	Full spectrum lights; 49-97% lower catches at treated sites in 1993 *versus* same sites in 1992; statistically significant decrease; three sites (*Orthosia hibisci, Protorthodes rufula, Perizoma curvilinea*) eliminated from site? Overall, moth diversity unaffected.	Crawford et al. 1993

Appendix 3: Toxicity in Non-target Lepidoptera			
Product	Species/Exposure	Observations	Reference
B.t.k. (NOS)	Micro-and Macro-Lepidoptera exposed to 89 BIU/ha (36 BIU/acre) in 50 acre plots of oak woodland in Rockbridge County, VA	Sampled in 1992 and 1993. Pre- and post (day 6 and 12) foliage samples from canopy, subcanopy and shrub-layer show reductions in the relative abundance of 12/19 most common taxa. 12/16 were micro-Lepidoptera. In 1992, larval abundance reduced on 3/5 *B.t.k.* sites in canopy and subcanopy. Reduction in micro-Lepidoptera in 4/5 sites in canopy and 3/5 sites in subcanopy. Uneven application accounted for variable effects. Two plots consistently showed the greatest effects. No differences observed in total numbers of Lepidoptera on foliage in treated sites, compared with control sites in 1993. Micro-Lepidoptera accounted for 95% of the individuals collected from foliage in 1992 and about 85% in 1993. 6/8 most common macro-Lepidotpera species trapped under burlap bands were reduced by treatment. Three of these species were nearly absent in treated plots (*Satyrium calanus, Malacosoma disstria, Orthosia rubescens*). Other less common species appeared to be significantly less on treated plots. *Dasychira obliquatc* was not affected apparently. Noctuidae also lower in 1993.	Peacock et al. 1994
B.t.k. (Foray 48B)	Gypsy Moth and non-targets lepidoptera (sampled in 1991-1992) exposed to 14.4 BIU/ha (36 BIU/acre) (sprayed in May 1991) on 24 50 acre plots in oak, hickory with pine, and blueberry shrub layer in and Grant and Pendleton Counties, WV	Four treatments: control; *B.t.* sprayed without gypsy moth; *B.t.* with gypsy moth; gypsy moth alone (defoliated). Total larval abundance reduced following *B.t.k.* application in 1991. No effects of *B.t.k.* and gypsy moth on several Lepidoptera. Short-term effects of *B.t.k.* on non-target lepidoptera are detrimental but longer term effects are beneficial. Minor effect on some species of lepidoptera consumed by bats (Noctuidae and Notodontidae).	Sample et al. 1996

Appendix 3: Toxicity in Non-target Lepidoptera			
Product	Species/Exposure	Observations	Reference
B.t.k. (Foray 48B)	Karner blue butterlfy (*Lycaeides melissa samuelis*) larvae (early and late instars) reared on wild lupine foliage treated in laboratory bioassay with *B.t.k.* at rate of 30-37 or 90 BIU/ha for 7 days. A concurrent laboratory bioassay involving gypsy moth 2nd instars on similarly treated white oak for 7 days.	Survival rates for Karner blue larvae were: 100% for controls, 27% at 30-37 BIU/ha treatment rate, and 14% at 90 BIU treatment rate. Survival rates for gypsy moth larvae were: 80% for controls; 33% for low-dose treatment, and 5% for high-dose treatment. Investigators conclude that the Karner blue is both phenologically and physiologically susceptible to *B.t.* used for gypsy moth suppression, although the larval generation at risk and extent of phenological overlap may vary from year to year.	Herms et al. 1997
B.t.k. (Dipel: wettable powder)	Mulberry silkworm (*Bombyx mori*) larvae exposed to laboratory concentrations of 1x10, $1x10^2$, $1x10^3$, $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$, or $1x10^9$ spore/mL applied to mulberry leaves	LC_{50} = 1.40x10 spores/L (larval instar I) LC_{50} = $4.20x10^2$ spores/L(larval instar II) LC_{50} = $1.0x10^3$ spores/L(larval instar III) LC_{50} = $2.0x10^5$ spores/L(larval instar IV) LC_{50} = $6.3x10^6$ spores/L(larval instar II) Larval mortality was dose-dependent with highest % mortality observed at highest concentrations of *B.t.* The highest % of mortality was observed in the early instars, compared with the later instars, and a longer incubation period was observed at the lower concentrations. The higher concentrations of *B.t.* were associated with decreased pupation, greater pupal mortality, increased incidences of malformed adult emergence and lower emergence of normal adults in all instars.	Jayanthi and Padmavathamma 1997

Appendix 3: Toxicity in Non-target Lepidoptera			
Product	Species/Exposure	Observations	Reference
B.t.k. (Foray 48B)	Swallowtail butterflies (*Papilio glaucus* and *Papilio canadensis*) and promethea moth (*Callosamia promethea*) (1st and 2nd instars of the three nontarget species) exposed to Foray 48B applied at a rate of 40 BIU/ha to individual trees using a *B.t.-dedicated* backpack sprayer to eliminate possibility of contamination from other insecticides. Larvae were placed on the tree at 0 or 1 day after spray and monitored for 7-8 days.	Significant differences in larval survival by day 5 between sprayed and control trees; nearly all larvae died or disappeared by day 8 from sprayed foliage. See text for additional details.	Johnson et al. 1995
B.t.k. (Foray 48B)	Long-term persistence field studies in which Foray 48B was applied at a rate of 40 BIU/ha to 5-year-old, 1-2 m high potted tulip trees which were randomly assigned to full sun or below-canopy locations in the field sites.	Tree survival was lower in the below-canopy locations, but the differences were not always significant. Toxicity toward early instar *P. glaucus* persisted for up to 30 days.	Johnson et al. 1995
Dipel 8AF	Laboratory bioassays equivalent to application rate of 89 BIU/ha.	18 species of lepidoptera native to U.S. 8 species of larvae (44%) evidenced significant mortality.	Peacock et al. 1998 See text and Tables 4-1 and 4-2 to additional details.
Foray 48B	Laboratory bioassays equivalent to application rate of 99 BIU/ha.	42 species of lepidoptera native to U.S. 27 species of larvae (61%) evidenced significant mortality.	
Foray 48F	Field study in which Foray 48F was applied at a rate of 40 BIU/acre in May of 1997 and 1998 to two forests susceptible to gypsy moth. Nontarget lepidoptera monitored in two pre-treatment year as well as in treatment years.	Larvae of three lepidopteran species were significantly decreased in treatment years: *Lambdina fervidaria* [geometrid], *Heterocampa guttivitta* [notodontid], and *Achatia distincta* [noctuid]. For 19 other species, larval counts were significantly higher in treatment years as were the total number of noctuids combined and the total number of all nontarget lepidopteran species combined.	Rastall et al. 2003
Dipel 6AF (12,000 IU/mg)	Applied aerially at 59 BIU/ha (ca. 24 BIU/acre).	Two non-target lepidoptera: *Incisalia fotis* (Desert Elfin butterfly) and *Callophrys sheridanii* (Sheridan's Hairstreak butterfly). Significant mortality in larvae that was dose-related. 3,473 cfu/mm^2 lead to nearly 80% mortality in 7 days.	Whaley et al. 1998

Appendix 3: Toxicity in Non-target Lepidoptera			
Product	Species/Exposure	Observations	Reference
B.t.k. (Dipel-HG) potency of 4320 IU/mg	Cinnabar moth (*Tyria jacobaeae*) larvae (1st - 5th instar) allowed to feed on tansy ragwort leaf pieces dipped in concentrations of 0, 0.24, 0.094, 0.295, 0.943, or 2.95 mg formulation/mL water (corresponding to field rates of 0, 2, 8, 25, or 250 BIU/ha); Cabbage looper (*Trichoplusia ni*) used as postive control.	LC_{50} = 26 BIU/ha (4th instar) (95% CI = 9.6-62 BIU/ha) LC_{50} = 19 BIU/ha (5th instar) (95% CI = 5.9-44 BIU/ha) LC_{50} = 16 BIU/ha (*Trichoplusia ni*) (95% CI = 5.6-30 BIU/ha) Treatment had little effect on 1st through 3rd instar survival) – LC_{50} values of 427 to 575 BIU/ha. See text for discussion.	James et al. 1993
B.t.k. (Dipel 2X)	Diamondback moth exposed to topical application	Direct dip LC_{50} >100 mg/mL Leaf dip LC_{50} = 0.014 mg/mL	Idris and Grafius 1993 Summarized in USDA 1995
B.t.k. HD-1 strain (Thuricide 32 LV)	White-marked tussock moth (*Orgyia leucostigma*) larvae (early 3rd instar) via dietary exposure	LC_{50} = 12 IU/mL diet (95% CI = 9-13 IU/mL)	Frankenhuyszen et al. 1992
B.t.k. HD-1 strain (Thuricide 32 LV)	Eastern hemlock looper (*Lambdina fiscellaria fiscellaria*) larvae (early 3rd instar) via dietary exposure	LC_{50} = 162 IU/mL diet (95% CI = 129-343 IU/mL)	Frankenhuyszen et al. 1992
B.t.k. HD-1 strain (Thuricide 32 LV)	Jack pine budworm (*Choristoneura pinus*) larvae via dietary exposure	LC_{50} = 145 IU/mL diet (95% CI = 121-169 IU/mL)	Frankenhuyszen et al. 1992
B.t.k. HD-1 strain (Thuricide 32 LV)	Western spruce budworm (*Choristoneura occidentalis*)larvae via dietary exposure	LC_{50} = 11 IU/mL diet (95% CI = 9-13 IU/mL)	Frankenhuyszen et al. 1992
B.t.k. HD-1 strain (Thuricide 32 LV)	Spruce budworm (*Choristoneura fumiferana*) larvae (early 4th instar) via dietary exposure	LC_{50} = 63 IU/mL diet (95% CI = 46-82 IU/mL)	Frankenhuyszen et al. 1992
B.t.k. (Thuricide 32 LV) (84 BIU/L	Spruce budworm (*Choristoneura fumiferana*) exposed via diet for 14 days	LC_{50} = 160 IU/mL diet (95% CI = 139-183 IU/mL)	Frankenhuyszen and Fast 1989
B.t.k. (Thuricide 32 LV) (84 BIU/L	Western spruce budworm (*Choristoneura occidentalis*) exposed via diet for 14 days	LC_{50} = 26 IU/mL diet (95% CI = 20-33 IU/mL)	Frankenhuyszen and Fast 1989

Appendix 4: Toxicity in Non-Target Terrestrial Insects Other Than Lepidoptera (sorted by insect order).			
Product	Species/Exposure	Observations	Reference
Coleoptera (Beatles)			
B.t.k. (Dipel 4L) []	Convergent lady beetle (*Hippodamia convergens* Guerin) adults only exposed to 9.4 or 18.7 L/ha Dipel 4L and water (1:3)	No significant mortality at 9.4 L/ha [79 BIU/ha] for up to 7 days. At 18.7 L/ha [158 BIU/ha], 13.4% mortality attributable to *B.t.k.* at 7-days post-exposure.	Haverty 1982
Note on Haverty (1982): Dipel 4L is not used in USDA programs. This is an oil based formulation with 32 BIU/gallon (http://www.greenbook.net/docs/LABEL/L16533.PDF) or 8.45 BIU/L. The only oil based formulation used in USDA programs is Dipel ES (64 BIU/gallon).			
B.t.k. CGA-237218	Ladybird beetles (*Coccinella septempunctata*), 5-days, dietary, 10^5, 10^7, 10^9 cfu/g food.	Concentrations characterized as 80 to 1400X ECC. No observation period beyond dosing period. No increase in mortality. Mortality in exposed beetles consistently less than controls. This is not discussed in study.	Winter et al. 1990 Thompson 1991a
NOTE: Winter et al. 1990 and Thompson 1991a have identical data. Appears to be the same study.			
Collembola (snow-fleas, springtails)			
Dipel 8L (oil based) as well as formulation (oil) blank	Microcosm study using Collembola: 1000X EEC – i.e., 20,289 I.U./cc OM in soil. Observations at weeks 2,3,4, and 6 after treatment.	Collembolan populations significantly decreased with both *B.t.k.* formulation and oil blank.	Addison and Holmes 1995
Dipel 8AF (aqueous) as well as unformulated *B.t.k.*		No effects on Collembolan populations.	
Dermaptera (earwigs)			
B.t.k. (Dipel WP)	Striped earwig (*Labidura riparia*) exposed to 10x label application rate	No mortality observed	Workman 1977 as summarized in USDA 1995

Appendix 4: Toxicity in Non-Target Terrestrial Insects Other Than Lepidoptera (sorted by insect order).			
Product	Species/Exposure	Observations	Reference
Diptera (flies)			
B.t.k. HD-1 (serovar 3a3b)	Laboratory bioassay in Mexican fruit fly (*Anastrepha ludens*).	Significant mortality from both pellet and supernatant preparations of *B.t.k.* in agar. Screening study using a variety of different *B.t.* strains to test for efficacy. Not directly useful for dose-response comparisons.	Robacker et al. 1996
Hemiptera (Bedbugs, aphids, cicadas)			
B.t.k. (Bactospeine WP) produced in the Netherlands	Spined soldier bug (*Podisus maculiventris*) (4^{th} instars and 7-day-old female adults) exposed to *B.t.k.* formulation (16,000 IU mg^{-1}) via ingestion for 48 hours	No adverse effects and no mortality observed at the highest dose tested (10,000 mg formulated material/L).	Mohaghegh et al. 2000
Hymenoptera (ants, bees, wasps, sawflies, chalcids, and ichneumons)			
Bees			
B.t.k. , Bactec Corp. 14.5 BIU per lb	Honey bees (*Apis mellifera*): Contact toxicity. 0, 7.7 , 15.4, and 23.2 µg/bee corresponding to 0.7, 1.4, and 2.1 lb/acre. Application rates correspond 1.73, 3.45, or 5.19 lb/ha which also corresponds to 25, 50, and 75 BIU/ha.	Mortality at 48 hours: BIU/ha Mortality Corrected 0: 7.17% 25 19% 12.7% 50 25% 19.2% 75 24.9% 19.1% See text for additional discussion. W1	Atkins 1991a [Atkins 1991b appears to be the same study but with a different MRID number.]
B.t.k. NOS	Honey bees	10-day LC 118 ug/bee (consumed)	MRID 435681-01 summarized but not referenced in U.S. EPA 1998
B.t.k. NOS	Honey bees	No significant effects at 10X field rate (NOS).	MRID 434917-02 summarized but not referenced in U.S. EPA 1998
Ants			
Foray 48F	Ants, various species. Field study involving 18 plots in Augusta County, VA. 16 BIU/ha (ca. 6.5 BIU/acre) in May 1997.	No substantial effects on ant populations: abundance, species richness, composition and diversity over a 3 year sampling period. A decrease of abundance was noted in the third year but was attributed to over-trapping.	Wang et al. 2000
Mantodea (mantids sometimes included with Dictyoptera/roaches)			

Appendix 4: Toxicity in Non-Target Terrestrial Insects Other Than Lepidoptera (sorted by insect order).			
Product	Species/Exposure	Observations	Reference
B.t.k. (Commercial formulation containing 18,000 IU/mg)	Chinese praying mantis (*Tenodera aridifolia sinensis*) exposed via consumption of cabbage looper larvae that had consumed *B.t.k.* for 15 hours in 150 µg/mL diet	No effect on mortality or survival	Yousten 1973
Neuroptera (antlions, lacewings, and Dobsonflies)			
Dipel, specified only as "technical powder". No BIU equivalents given.	Common green lacewing (*Chrsoperla carnea*) 0.1X, 1X, and 10X field application rate. Direct spray and residue exposure.	Increased mortality in high dose group but not significantly different from controls. Higher than expected mortality in control groups and high variability among replicates.	O'Leary 1990
B.t.k. (Dipel 4L)	Common green lacewing (*Chrysopa carnea* Stephens) adults and larvae exposed to 9.4 or 18.7 L/ha Dipel 4L and water (1:3)	Low mortality in larvae (2.1%) and adults (2.0%) at 9.4 L/ha [79 BIU/ha] for up to 7 days. At 18.7 L/ha [158 BIU/ha], mortality increased for both adults (5.3%) and larvae (6.7).	Haverty 1982
B.t.k. Biobit	Common green lacewing (*Chrsoperla carnea*), 9-days dietary, 4×10^4, 2×10^6, and 10^8 cfu/g feed.	No mortality in control group (0/30). Mortality in dosed groups of 3/30, 4/30, and 4/30. [Note: P-value of 0/30 vs 4/30 is 0.0562 using Fisher Exact test.]	Hoxter et al. 1990a
B.t.k. CGA-237218	Green lacewing (*Chrsoperla carnea*), 5-days dietary, 10^6, 10^7, and 10^8 cfu/g feed. 9-day post observation period	No dose-related increase in mortality. Mortality rates in dosed groups ranged from 3% (mid-dose) to 33% (low-dose). Mortality rates in control groups ranged from 23% to 37%.	Thompson 1991b
Omitted studies by Winter et al. 1991a, Hoxter and Smith 1991 on Delta BT. Cannot identify as *B.t.k.* Omitted Kirkland 1991, Nelson 1991b, and Palmer and Beavers 1993 studies on *B.t.* Abbott ABG-6305. This is *B.t.a.*			

Appendix 5: Toxicity of *B.t.k.* and *B.t.k.* Formulations to Fish.			
Product	Species/Exposure	Observations	Reference
Dipel Technical Material	Bluegill sunfish (n=30), 32 days, static renewal, at 2.87×10^7 cfu/L nominal (1.45×10^7 cfu/L measured)	No mortality, abnormal gross pathology, and no effects on body weight or length.	Christensen 1990c
Dipel Technical Material, 2.0×10^{10} cfu/g and 88,200 IU/mg.	Rainbow trout (n=30), 32 days, static renewal, at 2.87×10^7 cfu/L nominal (1.51×10^7 cfu/L measured). The nominal concentration of 2.87×10^7 cfu/L corresponds to 1.4 mg/L or 123,480 IU/L.	6/30 treated fish and 1/30 control fish died, most during the last 14 days of the study [*p*-value of 0.052 using Fisher Exact test]. Mortality attributed to aggression/competition for food in cloudy test solution. No abnormal gross pathology and no effects on body weight or length. [Water pH and dissolved oxygen were within normal limts.]	Christensen 1990d
Dipel Technical Material	Sheepshead minnow (n=52), 30 days, static renewal, at aqueous concentration of 2.87×10^{10} cfu/L and dietary concentration of 2.87×10^7 cfu/L.	Concentrations characterized as 100X and 1000x expected environmental concentrations (EEC). Four fish died. In one fish, body burden of *B.t.k.* was higher than anticipated based on aqueous and dietary concentrations – it is unclear how this determination was made. No inflamation or necrosis.	Christensen 1990g
B.t.k. Biobit	Rainbow trout (n=30), 31 days, at aqueous concentration of 3.67×10^{10} cfu/L and dietary concentration of 1.41×10^{10} cfu/g.	Aqueous and dietary concentrations characterized as 1000x and 40,0000x expected environmental concentrations (EEC). Decreased mean body length and weight in exposed fish. No other signs of toxicity.	Christensen 1990i
B.t.k. CGA-237218	Rainbow trout (n=30), 32 days, at a nominal aqueous concentration of 3.9×10^{10} cfu/L and dietary concentration of 1.52×10^{10} cfu/g	Concentrations in water and diet characterized as 500X and 200,000x EEC. 1/30 fish died during exposure. No *B.t.k.* found in dead fish. Two fish has gill lesions from which *B.t.k.* could be cultured. The concentration in gills was less than the concentration in water.	Christensen 1991c
B.t.k. CGA-237218	Sheepshead minnow (n=30), 30 days, at a nominal aqueous concentration of 7.8×10^7 cfu/L and dietary concentration of 1.56×10^{10} cfu/g	Concentrations in water and diet characterized as 50X and 200,000x EEC. No mortality. No signs of toxicity or infectivity.	Christensen 1991e

Appendix 5: Toxicity of *B.t.k.* and *B.t.k.* Formulations to Fish.			
Product	Species/Exposure	Observations	Reference
B.t.k. (wettable powder formulation manufactured in India)	Mosquito fish (*Gambussia affinis*) 10 fish/group exposed to 0, 200, 400, 600, 800, or 1000 mg/L for 96 hours. The formulation contained 2.5×10^{7} spores/mg. Thus, these doses correspond to 0, 5×10^{9}, 1×10^{10}, 1.5×10^{10}, 2×10^{10}, and 2.5×10^{10} spores/L.	No mortality observed. No signs of sublethal toxicity – i.e., no effects on swimming behavior, reflexes, general appearance, and gill movement.	Meher et al. 2002
B.t.k.	Rainbow trout, 96 hour exposure	$LC_{50} > 10$ mg/L	Mayer and Ellersieck, 1986
B.t.k.	Bluegill sunfish, 96 hour exposure	$LC_{50} = 95$ mg/L	Mayer and Ellersieck, 1986
B.t.k. as unformulated product in Foray 48B	Koi carp (*Cyprinus carpio*) exposed to 1x or 10x ECC via food and water in experimental tanks for 32 days	Small quantities of bacteria unrelated to *B.t.* were recovered from various fish organs; bacteria occurred predominantly in the intestine; *B.t.* found intermittently; some of the *B.t.* strains isolated were not the strain applied to the tank; sublethal effects observed in the treated fish were independent of *B.t.* recovery; sublethal adverse effects included significant decreases in plasma protein values and body weight.	Martin et al. 1997 **NOTE:** This is an abstract and the reported finding cannot be well evaluated. A full publication has not been encountered in the literature. See Section 4.1.3.1 for discussion.
B.t.k. technical material	Bluegill sunfish, 100x MEEC (maximum expected environmental concentration) in water and diet for 30 days	no evidence of pathogenicity	Abbott Labs 1992 **Note:** This is a non-detailed summary and cannot be well evaluted.
Omitted Bellantoni et al. 1991a,d on Delta BT. Cannot identify strain.			

Appendix 6: Toxicity of *B.t.k.* and *B.t.k.* Formulations to Aquatic Invertebrates (sorted by specified group – phylum, order, or subclass – followed by studies on mixed populations).			
Cladocera			
Dipel, NOS	*Daphnia magna*, 21-day static renewal, 0, 5, 50, and 100 mg/L. Constant aeration.	Increased BOD of test chambers at 50 and 100 mg/L. 21 Day EC_{50} of 14 mg/L based on immobilization. Delayed in time to first brood and number of young per adult at 5 mg/L.	Young 1990
B.t.k. CGA-237218 [Specified as containing 1.06×10^{11} cfu/g equivalent to 1.06×10^{8} cfu/mg].	*Daphnia magna*, 21-day static renewal. Measured concentrations of 0, 4.85×10^{7}, 1.57×10^{8}, 6.24×10^{8}, 1.77×10^{9}, 5.71×10^{9} cfu/L. Aeration not specified. These concentrations are equivalent to about 0, 0.45, 1.4, 5.9, 17, and 54 mg/L.	No daphnids survived at two highest concentrations. Decreased survival at three lower concentrations: 85% (low), 10% (mid), and 30% (high). Decrease significant only at mid-concentration group. No difference in reproduction at the two lower concentrations. Substantial decreases in dissolved oxygen at two highest concentrations [Table 1, p. 28/90].	Christensen 1991d
Copepoda			
B.t.k. technical material	*Amphiascus minutus* (copepod). 5, 50, and 500 mg/kg sediment for 10 days. (1×10^{5}, 1×10^{6}, and 1×10^{7} cfu/g sediment)	No adverse effects at any concentration on survival or reproduction. Number of offspring at 500 mg/kg was significantly greater than controls, probably due to the utilization of *B.t.k.* as a food source.	Chandler 1990b; Abbott Labs 1992
Glass Shrimp (Palaemonetes)			
Dipel technical material	Grass shrimp (n=60), 30-day static renewal, 100X EEC in water and food: 2.87×10^{9} cfu/L and 2.87×10^{9} cfu/g food.	One shrimp died in both exposed and control groups. No significant differences in body weight or length. No apparent adverse effects.	Christensen 1990h
B.t.k. CGA-237218	Grass shrimp (n=60), 30-day static renewal, dietary: 1.58×10^{10} cfu/g food. Concentration characterized as 200,000 EEC.	Mortality of 12/60 in treatment groups and 14/60 in control group. No effect on survival or growth. No signs of infectivity or pathogenicity.	Christensen 1991f

Appendix 6: Toxicity of *B.t.k.* and *B.t.k.* Formulations to Aquatic Invertebrates (sorted by specified group – phylum, order, or subclass – followed by studies on mixed populations).			
Glass Shrimp (Palaemonetes) *(continued)*			
B.t. technical material	Grass shrimp, 100x MEEC (maximum expected environmental concentration) in diet for 30 days	no adverse effects	Abbott Labs 1992 [appears to refer to Christensen 1990h]
Trichoptera			
B.t.k. (Dipel 64 AF)	Caddisfly (*Hydatophylas argus*) larvae exposed to aqueous flowable formulation applied to leaf disks treated with 20 IU/mL (maximum expected environmental concentration) or 20,000 IU/mL (1000x expected environmental concentration) for 2 days under flow-through conditions.	Treatment had no apparent effect on the palatability of the leaf disks; no significant differences among treatment levels with regard to leaf consumption; no mortality observed	Kreutzweiser and Capell 1996
Mixed Populations			
B.t.k. (Thuricide 32 LV containing 8.45 BIU/L)	Larvae of Simulidae, Chironomidae, Trichoptera, Megaloptera, and nymphs of Ephemeroptera and Plecoptera at continuous exposure to 4.3, 43, or 430 IU/mL. These concentrations correspond to 4300, 43,000, and 430,000 IU/L. Assuming a density of 1 for the formulation, 8.45 BIU/kg corresponds to 0.00012 mg/IU. Thus, the concentrations correspond to about 0.5 mg/L, 5 mg/L, and 50 mg/L.	Clear signs of toxicity observed only in *Simulium vittatum* (black fly) in which only 6 adults emerged at 430 IU/mL; possible signs of toxicity were observed in *Prosimulium fascum/mixtum* (black fly) in which survival was decreased at 43 and 430 IU/mL, compared with 4.3 IU/mL concentration and with the controls.	Eidt 1985

Appendix 6: Toxicity of *B.t.k.* and *B.t.k.* Formulations to Aquatic Invertebrates (sorted by specified group – phylum, order, or subclass – followed by studies on mixed populations).			
Mixed Populations *(continued)*			
B.t.k. (Dipel 8AF with potency of 16.9 BIU/L)	Ephemeroptera (mayflies) (6 taxa); Plecoptera (stoneflies) (3 taxa); Trichoptera (caddisflies) (4 taxa) exposed to maximum concentration of 600 IU/mL (considered to be 100x the expected environmental concentration in 50 cm of water resulting from direct over spray) for 24 hours in continuous flow-through bioassay	No significant mortality in 11 species after 9 days; average mortality of 30% in stoneflies (*Taeniopteryx nivalis*) after 9 days.	Kreutzweiser et al. 1992
B.t.k. (Dipel 8AF with potency of 16.9 BIU/L) About 0.00006 mg/BIU.	Ephemeroptera (mayflies) (6 taxa); Plecoptera (stoneflies) (3 taxa); Trichoptera (caddisflies) (4 taxa) exposed to maximum concentration of 600 IU/mL for 2.5 hours in outdoor stream channels to measure lethal and drift response. Exposure considered to be 100x the expected environmental concentration in 50 cm of water resulting from direct over spray.	No effect on invertebrate drift; by 1 hour after exposure, the % drift was slightly but not significantly higher ($p>0.05$), compared with controls, in 5 of 10 species; no effect on survival of drifted insects 1 hour after applications. 24-hour LC_{50} values >600 IU/mL (600,000/L or 36 mg/L). No mortality in four species of Ephemeroptera and three species of Trichoptera. 4-30% mortality in 3 species of Plecoptera, 2 species of Ephemeroptera, and one species of Trichoptera.	Kreutzweiser et al. 1992
B.t.k. (Dipel 64AF)	caddisflies, mayflies, stoneflies (12 taxa) exposed to 10x label application	Only the stonefly (*Leuctra tenuis*) was reduced at 4 days after treatment	Kreutzweiser et al. 1993. Summarized in USDA 1995

Appendix 6: Toxicity of *B.t.k.* and *B.t.k.* Formulations to Aquatic Invertebrates (sorted by specified group – phylum, order, or subclass – followed by studies on mixed populations).			
Mixed Populations *(continued)*			
B.t.k. (Dipel 64 AF)	Macro invertebrate community in a section of forest stream (Icewater Creek, Ontario) exposed to direct application of nominal concentration of 200 IU/mL (10x expected environmental concentration)	No significant effects on abundance of most benthic invertebrates; limited impact of *B.t.k.* application on the stream invertebrate community includes a slight increase in invertebrate drift density at 0.5 hour application and only at the site 10 m below the application point and the significant reduction of the stonefly (*L. tenuis*) (~70%) 4 days after application. Although the abundance of the stonefly remained considerably lower at the treated site, compared with the reference site, for at least 18 days, the difference was not significant.	Kreutzweiser et al. 1994
B.t.k.	50-5000 BIU/ha over streams.	No effect on benthic stream communities or insect emergence. Increased drift rates in mayfly (*Baetis sp*)	Richardson and Perrin 1994
B.t.k.	Field trial for control of the spruce budworm	No effects 28 days after treatment relative to 14 days prior to treatment in populations of a number of aquatic invertebrates: Amphipoda, Decapoda, Hydracarina, Hirudinea, Hydrozoa, Nematoda, Oligochaeta, Porifera, Pulmonata and Turbellaria.	Buckner et al. 1974
Omitted Bellantoni et al. 1991b,c on Delta BT. Cannot identify strain. Omitted Boeri 1991, *B.t.a.*			

Appendix G
Gypchek (Nucleopolyhedrosis Virus) Risk Assessment

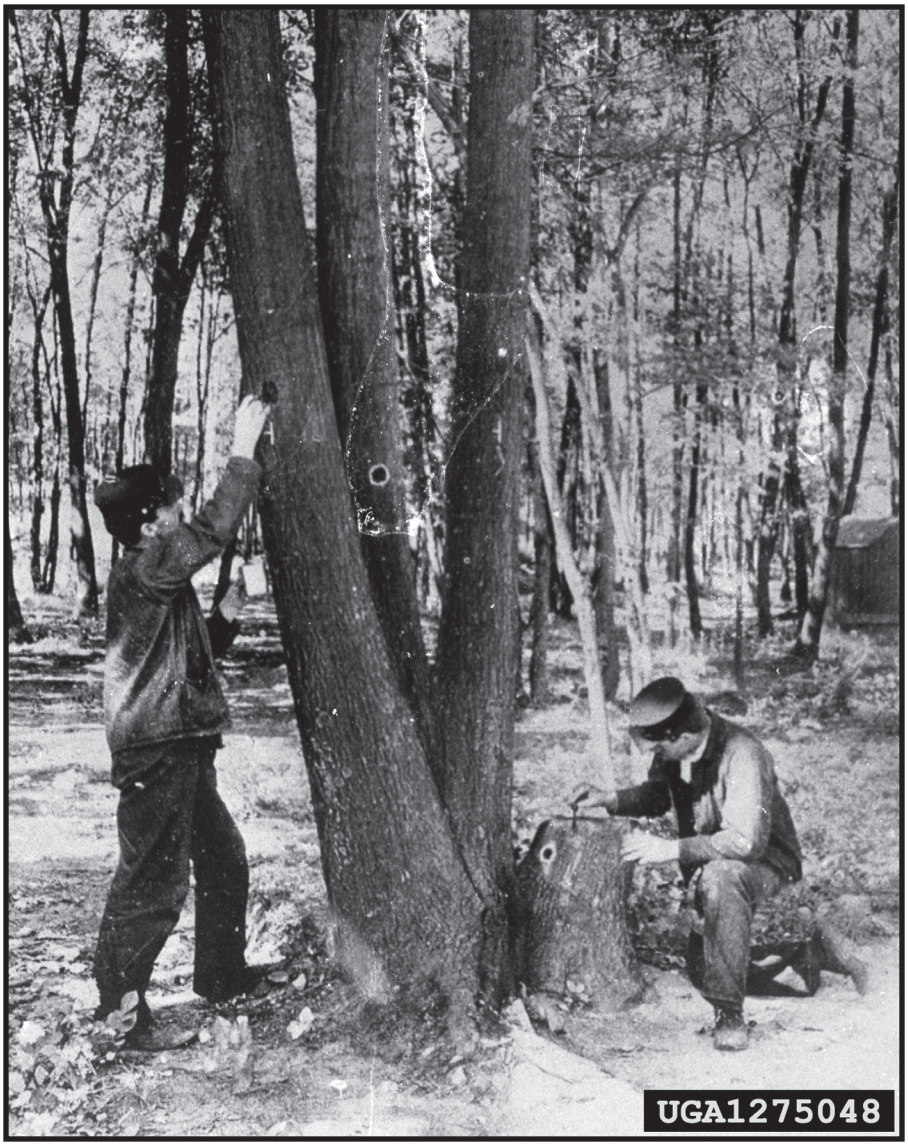

Figure G-1. Creosote was used in 1895 to treat gypsy moth egg masses.

Control/Eradication Agents for the Gypsy Moth - Human Health and Ecological Risk Assessment for Gypchek – a Nuclear Polyhedrosis Virus (NPV) FINAL REPORT

Prepared for:

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GENERAL ACRONYMS, ABBREVIATIONS, AND SYMBOLS

a.i.	active ingredient
A.U.	activity units
AEL	adverse-effect level
ACGIH	American Conference of Governmental Industrial Hygienists
bw	body weight
CBI	confidential business information
cm	centimeter
F	female
FS	Forest Service
g	gram
HQ	hazard quotient
kg	kilogram
L	liter
lb	pound
LC_{50}	lethal concentration, 50% kill
LD_{50}	lethal dose, 50% kill
LdNPV	*Lymantria dispar* (gypsy moth) nuclear polyhedrosis virus
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
mM	millimole
MNPV	multinucleocapsid nuclear polyhedrosis virus
MW	molecular weight
MOS	margin of safety
MSDS	material safety data sheet
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NPV	nuclear polyhedrosis virus
NRC	National Research Council
OB	occlusion body
OpNPV	*Orgyia pseudotsugata* (Douglas-fir tussock moth) nuclear polyhedrosis virus
OPPTS	Office of Pesticide Planning and Toxic Substances
PIBs	polyhedral inclusion bodies
ppm	parts per million
RED	reregistration eligibility decision
RfD	reference dose
TGAI	technical grade active ingredient
UF	uncertainty factor
U.S.	United States
U.S. EPA	U.S. Environmental Protection Agency
USDA	U.S. Department of Agriculture
>	greater than
≥	greater than or equal to
<	less than
≤	less than or equal to
=	equal to
≃	approximately equal to
~	approximately

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

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

Gypchek is a preparation of polyhedral inclusion bodies (PIBs) of the Gypsy moth nuclear polyhedrosis virus (LdNPV). Gypchek is a control agent for the gypsy moth developed and registered by the USDA Forest Service. This risk assessment is an evaluation of the potential consequences of using Gypchek and is an update to a previous risk assessment conducted for the Forest Service as part of the 1995 Final Environmental Impact Statement (FEIS) for the Cooperative Gypsy Moth Management Program. LdNPV is a naturally occurring baculovirus that is clearly pathogenic to gypsy moth larvae. There is no indication, however, that LdNPV is pathogenic or otherwise toxic to other species including other Lepidoptera humans. While the lack of toxicity displayed by Gypchek somewhat limits the quantitative expression of risk, very conservative estimates of exposure are below a plausible level of concern by factors of about 750 for humans, 1000 for terrestrial wildlife species, and 30,000 for aquatic species.

PROGRAM DESCRIPTION

The active ingredient in Gypchek is the gypsy moth nucleopolyhedrosis virus (NPV), commonly abbreviated as LdNPV. LdNPV is a naturally occurring baculovirus that is pathogenic to gypsy moth (*Lymantria dispar*) larvae causing a dissolution of tissues and the accumulation of polyhedral granules in the resultant fluid. The recommended application rate is 0.43 oz Gychek/acre for suppression and 1.08 oz Gypchek/acre for eradication. The application rate of 0.43 oz/acre corresponds to about 4×10^{11} PIB/acre and the application rate of 1.08 oz/acre corresponds to about 1×10^{12} PIB/acre. The production of Gypchek is very expensive and the application of this agent is currently limited to areas that are considered environmentally sensitive.

HUMAN HEALTH RISK ASSESSMENT

Hazard Identification – Gypchek does contain substantial amounts ($\geq$80% by weight) of gypsy moth larvae parts, including hairs which are known to cause skin and respiratory irritation in humans. Based on the available animal data, there is clear evidence that Gypchek can cause eye irritation. There is little indication that Gypchek is likely to cause dermal or respiratory irritation.

The toxicity data on LdNPV are reasonably complete and cover standard acute and chronic studies for systemic toxicity, standard assays for irritation of the skin and eyes, and basic pathogenicity studies required of most biological pesticides. While some new studies on eye irritation have been completed on Gypchek and LdNPV, most of the available studies are relatively old; they were conducted in the 1970's for the initial registration of Gypchek and most of the studies are unpublished. Nonetheless, these unpublished studies have been reviewed and accepted by U.S. EPA and have been re-reviewed in the preparation of this risk assessment. Also as with most pesticides, the toxicity data base on Gypchek is extremely limited for certain types of biological effects for which the U.S. EPA does not routinely require testing – i.e., immunotoxicity, endocrine effects, and neurotoxicity.

In terms of systemic toxicity or pathogenicity, there is not basis for asserting that Gypchek has the potential cause adverse effects at any exposure level. There is no indication that LdNPV is pathogenic in any mammalian species, even when the animal's immune function is compromised. Very high concentrations of Gypchek in the diet of rats – i.e., 500 mg/kg – have been associated with decreased food consumption and consequent loss of body weight but it is not clear that the effect was attributable to a toxic response to LdNPV since adverse effects, including mortality, were noted in the control group. Standard longer term toxicity studies in both rodents and dogs revealed no signs of toxicity.

Gypchek is typically applied with a carrier, either Carrier 038A or a lignosulfonate-molasses carrier and another product, Blankophor, may also be included in Gypchek applications. Toxicity data on these adjuvants are extremely limited. Carrier 038A is a proprietary surfactant formulation. Surfactants are soap-like materials that can have a spectrum of toxic effects, most of which involve irritation to biological membranes. This appears to be the case for Carrier 038A. Toxicity data on this material is scant. One available bioassay indicates that Carrier 038A is practically nontoxic to rainbow trout. Blankophor serves primarily to protect the LdNPV virus from sunlight but may also enhance the toxicity of the LdNPV to the gypsy moth. There is limited toxicity data on this compound that indicates a very low toxicity.

Exposure Assessment – Given the failure to identify any hazard associated with Gypchek and LdNPV, there is little basis for conducting a detailed exposure assessment for Gypchek. Gypchek does contain gypsy moth parts and these constituents, as with gypsy moth larvae themselves, have irritant effects in humans. The use of Gypchek, however, will not add substantially to exposures to gypsy moth parts in infested areas and will serve to reduce exposure to gypsy moth larvae by reducing larval populations.

Based on simply physical processes associated with the application of any pesticide, it is possible to construct any number of exposure scenarios for Gypchek. The current risk assessment 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.

Dose-Response Assessment – Because no systemic toxic effects can be qualitatively identified for any plausible routes of exposure (i.e., oral, dermal, or inhalation), the U.S. EPA has not derived either an acute or chronic RfD for Gypchek. While this is a reasonable approach, the current risk assessment derives a surrogate acute RfD of 26 mg/kg bw based on an experimental acute NOAEL of 2,600 mg/kg bw in rats and the application of an uncertainty factor of 100. This approach is taken simply to provide a more quantitative basis for comparing the extremely low risks associated with the application of Gypchek to the risks posed by other agents that may be used to control the gypsy moth.

Technical grade Gypchek is an eye irritant. While not quantitatively considered in this risk assessment, the distinction between the irritant properties of technical grade Gypchek and the lack of eye irritation with Gypchek formulations as applied in the field is emphasized in order to highlight areas in which prudent handling practices are likely to be most important.

Risk Characterization – There is no basis for asserting that any risk is plausible to either workers or members of the general public in 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 of about 50 to over 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.

ECOLOGICAL RISK ASSESSMENT

Hazard Identification – 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 any 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 essentially identical to those in mammals indicating that exposures to LdNPV at levels that are substantially higher than those likely to occur in the environment will not be associated with any adverse effects. Based bioassays of LdNPV on the large number of nontarget insect species and supported by the generally high species specificity of related baculoviruses, the hazard identification for LdNPV in nontarget insects is essentially identical to that in birds and mammals. There is no indication that adverse effects will be caused in nontarget insects at any level of exposure. Relatively few studies have been conducted in fish and aquatic invertebrates but these studies are consistent with studies in terrestrial species and indicate that effects on fish or aquatic invertebrates are unlikely. 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.

Exposure Assessment – In ground or aerial applications, it is likely that a large number of species could be exposed to Gypchek/LdNPV. The need for any formal risk assessment is questionable, however, because neither Gypchek nor LdNPV appear to cause systemic adverse effects. Nonetheless, in an attempt to provide some bases for comparing the potential risks of Gypchek to 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 mg Gypchek /kg bw to 3.2 mg Gypchek /kg bw. For aquatic organisms, concentrations are expressed in units of PIB/liter 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. A large number of other less extreme exposure assessments could be developed but these would not alter the assessment of risk since these extreme exposure assessments are substantially below any level of concern.

Dose-Response Assessment – Because no hazards can be identified for any species, a quantitative dose-response assessment is not required and no such assessments have been proposed by U.S. EPA and no quantitative dose-response assessments were used in the previous gypsy moth risk assessment for Gypchek. In order to provide a clear comparison of the risks of using Gypchek relative to other agents, dose-response assessments are proposed in the current risk assessment for both terrestrial mammals and aquatic species. For terrestrial mammals, the NOAEL of 2,600 mg/kg bw is used. This is the same NOAEL that served as the basis for the surrogate acute RfD in the human health risk assessment. For aquatic species, only NOEC values are available and the highest NOEC of 8×10^{9} PIB/L is used to characterize risk.

Risk Characterization – 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 NOEC and LOEC values cannot be defined – i.e., if an agent is not shown to cause an effect, the threshold exposure level 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.

1. INTRODUCTION

This risk assessment is an evaluation of the potential consequences of using Gypchek and is an update to a previous risk assessment conducted for the Forest Service as part of the 1995 Final Environmental Impact Statement (FEIS) for the Cooperative Gypsy Moth Management Program (Durkin et al. 1994; USDA 1995). The USDA Forest Service uses Gypchek in the control of the Gypsy moth (*Lymantria dispar*). Gypchek is a preparation of polyhedral inclusion bodies (PIBs) of the Gypsy moth nuclear polyhedrosis virus (NPV). Based on the recent re-registration eligibility decision (RED, U.S. EPA 1996) and a few more recent studies not cited in the RED, the present document provides risk assessments for human health effects and ecological effects of LdNPV to support an assessment of the environmental consequences of using Gypchek in Forest Service programs. In the re-registration process, the U.S. EPA (1996) combined data from the Gypsy Moth NPV (LdNPV) and a related virus, Tussock Moth NPV (OpNPV).

In addition to this introduction, this document includes a program description, a risk assessment for human health effects, and a risk assessment for ecological effects or effects on non-target wildlife species. Each of the two risk assessment chapters has four major sections, including an identification of the hazards associated with LdNPV, an assessment of potential exposure to the virus, 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.

Nonetheless, this risk assessment of LdNPV is qualitatively different in some ways from risk assessments of chemical agents. Because NPVs are biological organisms rather than chemicals, many standard physical and chemical properties used to characterize chemical compounds and estimate certain exposure parameters (e.g., SERA 2001) simply do not apply to LdNPV or other NPVs. More significant is the fact that most NPVs including LdNPV are highly host specific. LdNPV is pathogenic to the gypsy moth. In this species, LdNPV produces a well-characterized effect for which the most meaningful exposure metameter is clearly the number of active polyhedral inclusion bodies (PIBs). For other species, including humans, PIBs are a less meaningful measure of exposure because LdNPV does not appear to affect non-target species. Instead, the available information suggests that most adverse effects in non-target species associated with exposure to Gypchek are likely to be associated with insect parts in the commercial formulation.

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 (e.g., efficacy studies) but are focused on the information that most clearly impacts an assessment of risk. Most of the mammalian toxicology studies and some ecotoxicology and environmental fate studies are unpublished reports submitted to the U.S. EPA as part of the registration or re-registration of LpNPV. Full text copies of studies submitted to the U.S. EPA were kindly provided by U.S. EPA/OPP (n=81). These studies were reviewed and are discussed in this document.

This is a technical support document and it addresses some specialized technical areas. Nevertheless, an effort has been made to ensure that the document can be understood by individuals who do not have specialized training in the chemical and biological sciences. Certain technical concepts, methods, and terms common to most risk assessments are described in a separate document (SERA 2001). In addition, technical terms commonly used in this document and other risk assessments are defined in a glossary (SERA 2003) and more specialized terms are defined in the text as necessary.

2. PROGRAM DESCRIPTION

2.1. Overview

The active ingredient in Gypchek is the gypsy moth nucleopolyhedrosis virus (NPV), commonly abbreviated as LdNPV. LdNPV is a naturally occurring baculovirus that is pathogenic to gypsy moth larvae causing a dissolution of tissues and the accumulation of polyhedral granules in the resultant fluid. The recommended application rate is 0.43 oz Gychek/acre for suppression and 1.08 oz Gypchek/acre for eradication. The application rate of 0.43 oz/acre corresponds to about 4×10^{11} PIB/acre and the application rate of 1.08 oz/acre corresponds to about 1×10^{12} PIB/acre. The production of Gypchek is very expensive and the application of this agent is currently limited to areas that are considered environmentally sensitive.

2.2. Description and Commercial Formulation

Gypsy moth nucleopolyhedrosis virus (LdNPV) is a naturally occurring baculovirus that is usually important in bringing about the collapse of gypsy moth populations (Cook et al. 1997; Podgwaite 1979;Webb et al. 1999a,b). Gypchek is a powdered formulation of LdNPV developed and registered by USDA for control of the gypsy moth (Podgwaite 1999).

The active ingredient in Gypchek is about 12% (by weight) polyhedral inclusion bodies (PIB's) of LdNPV (USDA/FS 2003a). Some earlier preparations of Gypchek were about 20% LdNPV by weight (USDA/FS 19??c, MRID 00066097). [Note: Designations such as 19??c are used by U.S. EPA to identify submissions whose date is unclear. This designation is also used in this risk assessment for consistency with U.S. EPA.] The powder is produced by culturing and processing gypsy moth larvae infected with LdNPV (Lewis 1971; USDA/FS 1975). The average yield of PIB's in mass production is about 2×10^{9} PIB/larva (Lewis 1971) and the average weight of each PIB is about 3.66×10^{-12} grams (Adamson 1991). The active material is sometimes referred to as occulsion bodies (OBs) because the virus particles occluded, containing variable numbers of nucleocapsids (genetic material) within one protein envelope. The rest of the Gypchek formulation consists of gypsy moth parts (USDA/FS 19??a,b,c; USDA/FS 2003a). A similar product, Disparvirus, was developed in Canada (Nealis and Erb 1993). Gypchek causes polyhedrosis, a viral disease of insect larva, which is characterized by dissolution of tissues and the accumulation of polyhedral granules in the resultant fluid.

2.3. Application Methods, Rates, and Mixing

Gypchek is usually applied against first or second instars of the gypsy moth. Application rates or other measures of exposure to Gypchek can be expressed in various units, the most common of which are weight of formulation, weight of the virus PIBs, or counts of the polyhedral inclusion bodies. Based on the most recent product label (USDA/FS 2003a), the recommended application rate for aerial spray is 0.43 oz/acre for suppression and 1.08 oz/acre for eradication. For ground applications, a rate of 0.54 oz/acre is recommended. The current product label does not specify an application rate in PIBs per acre but does provide a reference value of 929.3 billion [9.293×10^{11}] PIB per ounce. The application rate of 0.43 oz/acre corresponds to about 4×10^{11} PIB/acre and the application rate of 1.08 oz/acre corresponds to about 1×10^{12} PIB/acre. This is very similar to the application rates considered in the 1995 risk assessment. In all applications, the Gypchek formulation is applied at particles sizes of 100–150 μ (Podgwaite 1994).

Gypchek is applied in a carrier. A number of different carriers and adjuvants have be evaluated for Gypchek including Carrier 244 from Novo Nordisk (Cunningham et al. 1996) and Blankophor BBH, supplied by Burlington Chemical Company (Thorpe et a. 1999; Webb et al. 1998, 1999a). Carrier 038 or a lignosulfonate-molasses formulation has been used with Gypchek (Podgwaite 1999). Both Carrier 038 and a lignosulfonate-molasses formulation are listed as agents that can be used with Gypchek on the current product label (USDA/FS 2003a). Carrier

038 is produced by Novo Nordisk (Webb et al. 1999b). A presumably related carrier, Carrier 038-A, is currently listed at the USDA Forest Service web site (http://www.dnr.state.wi.us/org/land/forestry/ fh/GM/). This carrier is produced by OMNOVA Solutions (1999) and is identified only as a proprietary mixture. No additional information on the constituents of Carrier 038 or Carrier 038-A have been located in the open literature or the U.S. EPA/OPP FIFRA files.

Applications of Gypchek vary depending on the carrier used. For Carrier 038, 0.95 gallons of the carrier are mixed with a small amount of water (0.05 gal.) and 6.4 grams of Gypchek. For the lignosulfonate-molasses carrier, 1.7 gallons of water are mixed with 1 lb of Lignosite AN, 0.26 lb of feed-grade molasses,0.04 gallons of Bond, and 15.9 grams of Gypchek (USDA/FS 2003a).

2.4. Use Statistics

Gypchek was applied to only 53,034acres – about 6600 acres per year between 1995 and 2003 (Table 2-1). As indicated in Table 2-1, this figure does not include the number of acres that were treated twice. Including these repeated applications, a total of 54,034 acres were treated between 1995 and 2003 (Onken 2004).

As noted by Podgwaite (1999), the application of Gypchek is very expensive and is limited to areas that are considered environmentally sensitive. Gypchek is highly specific to the gypsy moth and there is no indication that LdNPV will effect any nontarget species (Sections 3.1 and 4.1).

TABLE 2-1: Use of Gypchek from 1995 to 2001 for Suppression, Eradication, and Slow the Spread*

	1995	1996	1997	1998	1999	2000	2001	2002	2003	Total (acres)
Suppression	2,127	791	4,367	3,956	2,306	5,882	2,280	4,794	10,015	36,518
Eradication	0	0	0	2,122	5,254	0	0	0	0	7,376
Slow the Spread	262	0	374	0	500	0	0	0	8,004	9,140
Total	2,389	791	4,741	6,078	8,060	5,882	2,280	4,794	18,019	53,034

*Source: ***GMDigest,*** Morgantown, WV (http://fhpr8.srs.fs.fed.us/wv/gmdigest/gmdigest.html). Does not include areas that were treated twice.

3. Human Health Risk Assessment

3.1. HAZARD IDENTIFICATION

3.1.1. Overview

LdNPV is a naturally occurring baculovirus that is clearly pathogenic to gypsy moth larvae. There is no indication, however, that LdNPV is pathogenic to other species, including humans or other mammals. Gypchek, the commercial formulation of LdNPV, is produced by culturing infected gypsy moth larvae and Gypchek does contain substantial amounts (>80% by weight) of gypsy moth larvae parts, including hairs which are known to cause skin and respiratory irritation in humans. Based on the available animal data, there is clear evidence that Gypchek can cause eye irritation. There is little indication that Gypchek is likely to cause dermal or respiratory irritation.

Information on the toxicity data of LdNPV is reasonably complete and covers standard acute and chronic studies for systemic toxicity, standard assays for irritation of the skin and eyes, basic pathogenicity studies required of most biological pesticides. While some new studies on eye irritation have been completed on Gypchek and LdNPV, most of these studies are relatively old, being conducted in the 1970's for the initial registration of Gypchek and most of the studies are unpublished. Nonetheless, these unpublished studies have been reviewed and accepted by U.S. EPA and have been re-reviewed in the preparation of this risk assessment. Also as with most pesticides, the toxicity data base on Gypchek is extremely limited for certain types of biological effects for which the U.S. EPA does not routinely require testing – i.e., immunotoxicity, endocrine effects, and neurotoxicity.

There is no indication that LdNPV is pathogenic in any mammalian species, even when the animal's immune function is compromised. Very high concentrations of Gypchek in the diet of rats – i.e., 500 mg/kg – have been associated with decreased food consumption and consequent loss of body weight but it is not clear that the effect was attributable to a toxic response to LdNPV since adverse effects, including mortality, were noted in the control group. Standard longer term toxicity studies in both rodents and dogs have not identified adverse effects at any dose level tested.

Gypchek is typically applied with a carrier (Section 2). Toxicity data on the adjuvants are extremely limited. Carrier 038A is a proprietary surfactant formulation. Surfactants are soap-like materials that can have a spectrum of toxic effects, most of which involve irritation to biological membranes. This appears to be the case for Carrier 038A as well as many household soaps. Toxicity data on Carrier 038A is scant. One available bioassay indicates that the material is practically nontoxic to rainbow trout. Blankophor serves primarily to protect the LdNPV virus from sunlight but may also enhance the toxicity of the LdNPV to the gypsy moth. There is some limited toxicity data on this compound that indicates a very low toxicity.

3.1.2. Epidemiology Studies and Other Human Data

Epidemiology studies regarding health effects in humans after exposure to LdNPV were not located in the available literature. Gypchek contains substantial amounts of gypsy moth larvae parts and exposure to gypsy moth larvae has been associated with dermal and respiratory effects in humans (Durkin et al. 1995). Based on the available animal data, it is plausible that exposure to Gypchek could be associated with ocular irritation in humans (Section 3.1.11). The plausibility of respiratory irritation (Section 3.1.13) or dermal irritation (Section 3.1.11) is less clear.

3.1.3. Mechanism of Action (Persistence and Pathogenicity)

As discussed in the following subsections, LdNPV has been subject to a large number of relatively standard toxicity studies and there is no indication that LdNPV exposures are pathogenic in mammals. In addition, as detailed further in Section 4.1, LdNPV appears to be highly specific to the gypsy moth and does not appear to be pathogenic to other species. In addition, a series of experiments were conducted to determine if NPV could infect or otherwise affect mice immunosuppressed with cyclophosphamide, thymectomy, or anti-lymphocyte serum and guinea pigs immunosuppressed with cortisone or cobra venom factor. No lesions, histopathological changes, or signs of infection associated with treatment were noted (Shope 1976; Shope and others 1977). Circulating antibodies to the insect viral subfractions have not been observed in laboratory workers (Mazzone et al. 1976; Tignor et al. 1976). Thus, there is no basis for asserting that LdNPV poses a risk of pathogenicity in humans.

Persistence in lung tissue has been examined in a study submitted to the U.S. EPA by the U.S. Forest Service. Several summaries of this study are available but are poorly documented (USDA/FS 19??d, MRID 00066105; USDA/FS 19??g, MRID 00060701; USDA/FS 1975?, MRID 00090598). Only one of these studies, MRID 00066105, is explicitly cited in the U.S. EPA (1996) although a later submission, MRID 00090598, gives a somewhat fuller description of the study. As indicated in Appendix 1, rats were exposed to LdNPV via inhalation for 1 hour at a concentration of 6.12 ± 2.087 mg/L ($= 4.04 \times 10^{8} \pm 1.38 \times 10^{8}$ PIBs/L) and sacrificed 1, 7, or 14 days after exposure. Recovery of LdNPV from the lung, relative to amounts recovered immediately after exposure, were about 96% at day1, 68% at day 7, and 18% at day 14. Assuming first-order clearance, this corresponds to a clearance rate of 0.13 $days^{-1}$ or a halftime of about 5 days.

3.1.4. Acute Oral Toxicity

The U.S. EPA requires standard acute oral toxicity studies for the registration of most pesticides, including Gypchek. For microbial pesticides, additional requirements include assays for pathogenicity. The standard assays involving LdNPV or Gypchek are summarized in Appendix 1. A large number of studies have been submitted to U.S. EPA. As detailed in Appendix 1, many of these are duplicate submissions or submissions of preliminary results. Some of these refer to the test agent as *P. dispar* NPV, referring to *Porthetria dispar*, a former designation for the gypsy moth. Thus, *P. dispar* NPV is identical to LdNPV.

A single dose of LdNPV at 400 mg was not associated with any adverse effects in male or female rats over a 30-day observation period (Terrell and Parke 1976a,b). At a somewhat higher dose, 500 mg per rat, a transient (2 week) but statistically significant decrease was noted in body weights over a 35-day observation period (Terrell et al. 1976c). This effect was associated with decreased food consumption. As noted in Appendix 1, mortality was noted in both control (8/20) and treated (3/20) animals. Thus, it appears that the health of the animals may have been compromised by factors other than treatment with LdNPV. As noted above, no effects were seen in immunosuppressed mice at a dose of 0.02 g/mouse over a 21-day observation period (Shope et al. 1975, 1977). Hart and coworkers (Hart 1976; Hart and Thornett 1975a,c) also observed no signs of toxicity or pathogenicity in groups of 20 to 30 rats after single gavage doses of up to 1 mL of a 4×10^{10} solution of LdNVP per rat. The U.S. EPA (1986) indicates an additional acute oral/pathogenicity study (MRID 41738701) is available for LdNPV. This study, however, involved exposures to OpNPV and not LdNPV.]

3.1.5. Subchronic or Chronic Systemic Toxic Effects

No recent studies have been conducted on the subchronic or chronic toxicity of Gypchek. As detailed in Appendix 1, two standard longer term toxicity studies are available on Gypchek: a 90-day subchronic feeding study in dogs (Hart 1975a) and a two-year chronic feeding study in rats (Hart 1975b). Both of these studies were submitted for the initial registration of Gypchek and have been reviewed by U.S. EPA (1996) and accepted as supplemental in the reregistration of both Gypchek and TM-Biocontrol.

In the subchronic study, purebred beagles were given LdNPV in the diet at concentrations that resulted in average daily doses of 0, 10^7, 10^8, or 10^9 OB of LdNPV/dog for 90 days. These doses correspond to Gypchek doses of 0, 1.8, 18, or 180 mg formulation/dog. The terminal body weights reported in the study were 9.5 kg for the low dose group, 11.1 kg for the middle dose group, and 10.3 kg for the high dose group. These doses expressed in mg Gypchek/kg bw equal 0.2 mg/kg for the low dose group, 1.6 mg/kg for the middle dose group, and 17 mg/kg for the high dose group. Each dog was observed at least once daily for gross effects. Standard hematology, clinical biochemistry, and urinalysis were conducted on each animal at or before the start of exposure and at 2, 4, and 6 months after the start of exposure. After sacrifice, standard examinations were conducted for signs of gross pathology or histopathology. No treatment related effects were observed (Hart 1975a).

In the chronic study, Dublin (Sprague-Dawley derived) rats were given LdNPV in chow at levels that resulted in daily doses of 10^7 or 10^8 OB/rat for 2 years. This exposure corresponded to Gypchek daily doses of 1.8 or 18 mg/rat. The average terminal body weights (both sexes combined) was approximately 400 g. Thus, the dose rate was 4.5 or 45 mg Gypchek/kg bw. Each of the treated and control groups consisted of 50 males and 50 females. Observations included body weight, food consumption, gross signs of toxicity, and pathology. No increased mortality was observed and no pathological changes were attributed to treatment (Hart 1975b).

As discussed in Section 4.1.2.1 and also summarized in Appendix 1, mammalian feeding studies have been conducted on various mammalian predators of the gypsy moth (Lautenschlager et al. 1977) but the exposure data from this study is not sufficiently detailed to permit a clear assessment of the actual doses that were used. Nonetheless, this study is consistent with the above standard studies in that no signs of toxicity were observed in any species.

3.1.6. Effects on Nervous System

A *neurotoxicant* is 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* is critical because it distinguishes agents that act directly on the nervous system *(direct neurotoxicants*) from those agents that might produce neurologic effects that are secondary to other forms of toxicity (*indirect neurotoxicants*). Virtually any agent (microbial or chemical) will cause signs of neurotoxicity in severely poisoned animals and thus can be classified as an indirect neurotoxicant.

Studies designed specifically to detect impairments in motor, sensory, or cognitive functions in mammals exposed to Gypchek or purified preparations of LdNPV have not been encountered in the open literature or in submissions to U.S. EPA. The U.S. EPA/OPTS (2003) has standard protocols for a number of types of neurotoxicity studies including a neurotoxicity screening battery (Guideline 870.6200), acute and 28-day delayed neurotoxicity of organophosphorus substances (Guideline 870.6100). Neither of these types of studies have been conducted on Gypchek. Further, the RED for LdNPV (U.S. EPA 1996) does not specifically discuss the potential for neurologic effects.

As summarized in Appendix 1, one early study on Gypchek, Terrell et al. (1976c), reports symptoms that are consistent either with either direct or indirect neurotoxicity – i.e., piloerection and decreased locomotor activity. These effects, however, occurred in both exposed and control animals. Based on both the acute and longer-term studies on Gypchek, there is no indication that exposure to LdNPV will be associated with either direct or indirect signs of neurotoxicity.

3.1.7. Effects on Immune System

With LdNPV or any other biological agent that may be pathogenic, the response of or pathological activity in immunocompromised animals – i.e., animals with impaired immune function – is a concern. In addition, some chemical or biological agents may act as immunotoxicants – i.e., chemical agents that disrupt the function of the immune system. Two general types of immunotoxic 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).

As summarized in Appendix 1, Shope et al. (1975) assayed the effects of LdNPV on normal and immunosuppressed animals by several routes of exposure: oral intubation, dermal application, ocular or intranasal installation, and footpad inoculation. The dermal studies were conducted on guinea pigs and other studies were conducted in mice. Differences in responses were observed between immunocompetent animals and immunosuppressed animals but these differences are attributable to the immunosuppressive agents rather than to any increased toxicity of LdNPV. Specifically, immunocompetent guinea pigs exhibited a greater skin irritant response to LdNPV than did immunosuppressed guinea pigs, indicating a general allergic reaction to the LdNPV in which a greater response in immunocompetent individuals would be expected. In mice, immunocompetent individuals evidenced a greater antibody titre than did immunosuppressed individuals after both oral exposure and intranasal installation (Shope et al. 1975). Again, this difference in response between immunocompetent and immunosuppressed mice would be expected after exposure to any antigenic material. In mice treated by footpad inoculation, secondary bacterial infections were noted. The study does not specify whether or not there were any differences in the incidence of bacterial infections between immunocompetent and immunosuppressed mice. Based on this study, the lack of marked dermal irritation (Section 3.1.11) and the low acute and chronic systemic toxicity of LdNPV (Sections 3.1.4 and 3.1.5), the U.S. EPA (1996) elected not to require additional testing on the immunologic effects of LdNPV.

3.1.8. Effects on Endocrine System

In terms of functional effects that have important public health implications, effects on endocrine function would be expressed as diminished or abnormal reproductive performance. As discussed in the following section (Section 3.1.9), however, very limited data are available on the reproductive effects of LdNPV. The potential for direct endocrine effects are typically assessed by various mechanistic assays (Durkin and Diamond 2002). LdNPV or other related NPV have not been tested for activity as an agonists or antagonists of the major hormone systems (e.g., estrogen, androgen, thyroid hormone). In the re-registration review for LdNPV, the U.S. EPA (1996) does not discuss the potential for effects on endocrine function. Thus, in the absence of direct experimental data on endocrine function or related toxicity studies that might be useful for assessing effects on endocrine function, no definitive hazard identification is possible. This does not imply that a risk is plausible. To the contrary, most endocrine active agents are synthetic

organic chemicals that mimic or otherwise interfere with the function of naturally occurring hormones. There is no basis for asserting that LdNPV is likely to have such an effect.

3.1.9. Reproductive and Teratogenic Effects

A number of standard tests for reproductive effects – i.e., effects on fertility – as well as tests for the potential to cause birth defects – i.e., teratogenicity – are available and are often required for pesticides. Examples of protocols for such tests are available from the U.S. EPA's web site: http://www.epa.gov/OPPTS_Harmonized/. These tests have not been required for LdNPV or OpNPV by the U.S. EPA (1996).

The only available information on the reproductive effects of LdNPV is the early study by Lautenschlager et al. (1977). This study reports no effects on reproduction in mice after they were fed diets containing LdNPV over a 20 day period. In the treated group, consisting of 8 males and 9 females, 5 litters with a total of 20 young were produced. In the control group, consisting of 10 males and 10 females, only 1 litter with 4 young was produced. While all exposures were dietary, the exposure regime was complex consisting of gypsy moth larvae infected with LdNPV, followed by a purified formulation of LdNPV, that was in turn followed by a diet containing a spray preparation of LdNPV. In any event, this study does provide a basis for asserting that relatively prolonged exposures to LdNPV did not cause adverse reproductive effects in mice.

3.1.10. Carcinogenicity and Mutagenicity

The two-year chronic feeding study in rats (Hart 1975b), which is discussed in Section 3.1.5 and summarized further in Appendix 1, is a standard *in vivo* assay for both chronic toxicity and carcinogenicity. As noted in Appendix 1, no increase in the incidence of tumors was noted in this study. This is the only long term study that is appropriate for assessing the potential carcinogenic effects of LdNPV.

3.1.11. Irritation (Effects on the Skin and Eyes)

LdNPV does not appear to be a marked skin irritant. As summarized in Appendix 1, relatively standard assays for dermal irritation noted no dermal irritation (Hart and Thornett 1975b,d,e; Becker and Parke 1976d) and, based on these studies, the U.S. EPA (1996) has classified LdNPV as *not a dermal irritant* (Category IV) (U.S. EPA 1996, p. 13).

The U.S. EPA (1996) has classified LdNPV as a Category I Eye Irritant – i.e., irritation with corneal involvement not cleared by day 14 after treatment. While the U.S. EPA (1996) cites many of the studies included in Appendix 1 in support of this determination, some studies (e.g., Hart and Thronett 1975f; Becker and Parke 1976c) noted little or only slight irritation. The most severe irritation and the only study consistent with the Category I designation is the study by Imlay and Terrell (1978) in which rabbits did evidence irritation with corneal opacity and conjunctival irritation that persisted through day 14 after treatment. This effect was seen, however, only in animals whose eyes were not washed at all after the instillation of a LdNPV formulation – i.e., Group 4 from the Imlay and Terrell 1978 study as summarized in Appendix 1. In other groups of rabbits whose eyes were flushed after treatment, signs of eye irritation were evident but much less severe.

Subsequent to the RED (U.S. EPA 1996), the Forest Service funded two studies on the ocular irritation of Gypchek, the commercial formulation of LdNPV. One study used the commercial formulation (Kuhn 1997a) and the other study used an aqueous solution at twice the anticipated field concentration (Kuhn 1997b). Both studies identify the test material as a 3.65×10^{10} PIBs/g LdNPV preparation [Lot GR-14A], a wettable powder. The study by Kuhn (1997a) characterizes the applied material as a "*Gypchek TGAI*", presumably referring to technical grade active

ingredient (i.e., the mixture of virus, insect parts and other ingredients). The study by Kuhn (1997b) characterizes the applied material as a "*Gypchek Solution 2X*", presumably indicating that the test solution was diluted to a concentration that is twice that used in field applications. Kuhn (1997b) does not specify the actual concentration of the test solution. In a letter of clarification to the U.S. EPA, Kuhn (1997c) indicates that the *2X* solution was a concentration of 2.92 mg technical product/mL. This dose is characterized as twice the field concentration based on a letter from Podgwaite (1996) indicating that the batch of Gypchek tested by Kuhn (1997a,b) would be diluted to 2×10^{11} PIBs/gallon and that this would correspond to 1.45 mg/mL.

In both studies, New Zealand White rabbits were dosed with 0.1 mL by volume of the test substance which was placed into the right eye of each of six males and six females. In the *TGAI* study (Kuhn 1997a), the eyes were washed for 1 minute beginning 30 seconds after treatment in three each of the males and females. None of the eyes were washed in the 2X study (Kuhn 1997b). The rabbits were examined at 1, 24, 48, and 72 hours as well as 4, 7, 10, 14, and 17 days after treatment.

In the *TGAI* study (Kuhn 1997a), the maximum average irritation score was 5.3 after 1 hour (minimally irritating) in the washed eyes and the maximum irritation score was 37.3 (moderately irritating) in the unwashed eyes. All effects cleared by day 17 after exposure. Based on U.S. EPA's classification scheme for ocular irritation, Kuhn (1997a) characterized the LdNPV preparation as Category II for non-washed eyes and Category IV for washed eyes. In the 2X study, no indication of eye irritation was noted and the test substance was assigned to Category IV, no or minimal effects.

Thus, while it is clear that LdNPV does have the potential to cause severe eye irritation, as demonstrated in the study by Imlay and Terrell (1978), it is less clear that such effects will be evident in the normal use of Gypchek with prudent use of protective measures to limit exposure to the eyes and to clean contaminated eyes in the event of unintended ocular exposure. This is discussed further in the risk characterization (Section 3.4).

3.1.12. Systemic Toxic Effects from Parenteral Exposure

Parenteral exposures involving injecting a substance into animal, typically into a vein (i.v.) or into the abdominal cavity (intraperitoneal or i.p. administration). These studies are used primarily as qualitative screening tools to assess general toxicity for both biological and chemical agents as well as pathogenicity and infectivity for biological agents. Two studies are listed in the U.S. EPA (1996) RED: Terrell and Parke 1976c and Terrell and Parke 1976d. Both of these studies appear to be identical, indicating no mortality or signs of toxicity in mice after a single intraperitoneal dose of about 125 mg/kg bw (Appendix 1).

3.1.13. Respiratory Effects and Inhalation Exposures

Two standard acute inhalation studies have been conducted on Gypchek and are summarized in Appendix 1. Neither of these studies gives a direct indication of toxicity. In one study, no overt signs of toxicity were observed in a group of 10 male rats exposed to 6.12 mg/L Gypchek for 1 hour. During exposure, the rats were inactive and had closed eyes and labored respiration. Examinations for lung and trachea pathology 1, 7, and 14 days after recovery revealed no effects attributable to exposure (Brown 1976). In the other inhalation study, rats were subjected to heads only exposure to avoid ingestion during grooming (Thornett 1975). The test material was a white dust with $1.76 \cdot 10^{11}$ OB/g. The exposure concentrations ranged from 0.028 to 0.81 mg/L. No signs of toxicity were observed in any of the rats during exposure or upon necropsy.

As noted in Section 3.1.7, Shope et al. (1975) used intranasal instillations to assess differences in response between immunosuppressed and immunocompetent mice. Intranasal instillations are

sometimes used as surrogates for inhalation exposures, particularly for biological agents that have a low order of toxicity and pathogenicity. Other than expected changes in immunocompetent mice associated with exposure to a foreign protein, no signs of pathogenicity were apparent.

3.1.14. Impurities and Contaminants

As indicated in Section 2.2, Gypchek is produced by culturing and processing gypsy moth larvae infected with LdNPV (Lewis 1971; USDA/FS 1975). The main contaminant in Gypchek is gypsy moth parts, which account for a substantial proportion (80-88%) by weight of the formulation (USDA/FS 19??a,b,c; USDA/FS 2003). In response to the potential for Gypchek to become contaminated with bacteria, a quality control program has been developed to ensure that batch preparations of NPV do not contain harmful bacteria (Podgwaite and Bruen 1978). The program consists of tests to determine bacterial counts of total aerobes, anaerobes, and bacterial spores; an enumeration of total and fecal coliform bacteria, assays for primary pathogens (that is, *Salmonella*, *Shigella*, *Vibrio*, *Streptococcus*, *Staphylococcus*, and *Clostridium*) and an *in vivo* pathogenicity test in mice. These tests are performed on each batch of Gypchek before it is used.

3.1.15. Inerts and Adjuvants

As indicated in Section 2.3, Gypchek is typically applied with a carrier, either Carrier 038A or a lignosulfonate-molasses carrier (Web et al. 1999c). Another product, Blankophor, may also be included in Gypchek applications to enhance the persistence and activity of LdNPV (Thorpe et al. 1999; Webb et al. 1999a,b).

Carrier 038A is an aqueous surfactant mixture consisting of 58.5% water and 41.5% proprietary surfactant mixture (Omnova Solutions 1999). Further details on the nature of the surfactant mixture are not available. The MSDS for Carrier 038A indicates that the surfactant mixture may cause mild to moderate eye, skin, and respiratory tract irritation. This is true for most surfactants, including household soaps, which may disrupt the lipid structure in biological membranes including those of the skin, eyes, and respiratory tract. The only specific information of the toxicity of Carrier 38A is a standard acute toxicity study in rainbow trout (Drottar and Krueger 2001) in which the 96-hour LC_{50} value was 914 mg/L with a corresponding NOEC of 600 mg/L. Based on the categorization system currently used by U.S. EPA/EFED (2001), Carrier 038A would be classified as practically nontoxic to rainbow trout.

Blankophor is the common or trade name for the disodium salt of 2,2'-stilbendisulfonic acid, 4,4'-bis((4-anilino- 6-morpholino-s-triazin-2-yl)amino) (NIOSH 2003). The toxicity data available on this compound indicates that the compound has a very low acute oral toxicity with reported LD_{50} values in excess of 80,000 mg/kg. In repeated dose skin exposures in rats at a dose of 21,000 mg/kg bw, changes were seen in kidney and serum. This study is summarized by NIOSH (2003) and is a 1966 study from the Bulgarian literature. Blankophor serves primarily to protect the LdNPV virus from sunlight but may also enhance the toxicity of the LdNPV to the gypsy moth (Thorpe et al. 1999). The U.S. EPA is in the process of registering Blankophor as a new pesticide inert (www.bnckay.com/inerts.htm).

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview

Because adverse effects associated with Gypchek or LdNPV, there is little basis for conducting a detailed exposure assessment for Gypchek. Gypchek does contain gypsy moth parts and these constituents, as with gypsy moth larvae themselves, have irritant effects in humans. The use of Gypchek, however, will not add substantially to exposures to gypsy moth parts in infested areas and will serve to reduce 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 current risk assessment 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.

3.2.2. LdNPV and Gypsy Moth Parts in Gypchek

In the re-registration of both LdNPV and OpNPV, the related virus used to control the Douglas-fir Tussock moth, the U.S. EPA (1996) determinated that formal exposure assessments for the general public and workers were not required. Two reasons for this decision are given. First, there is essentially no reason to assert that any adverse effects are plausible, and, as subsequently detailed in section 3.3, there is no standard dose-response assessment. In other words, there is no indication that LdNPV will cause systemic adverse effects; therefore, a formal exposure assessment would serve little purpose.

Secondly, the use of LdNPV to control gypsy moth populations is likely to reduce rather than increase exposure to the insect parts that are in Gypchek preparations:

> *Spraying of the PIBs of OpNPV and LdNPV will not significantly increase exposure to larval hairs, microbes, or other by-products that occur in the preparation of the ai's* [active ingredients]. *Pest densities that necessitate spraying have a natural high background of these factors; moreover, dilution of the ai's in the spraying preparation and its sticking to the forest foliage reduce the likelihood of exposure to a negligible level.* (U.S. EPA 1996, p. 17)

In other words, the use of either LdNPV will not increase exposure to both the viruses in these products and the insects that they control.

The potential for Gypchek to reduce exposure to both the LpNPV and the moth larvae can be discussed in some detail. As summarized in Section 2.2, the application rates for Gypchek range from $4 \cdot 10^{11}$ PIB/acre per application to $1 \cdot 10^{12}$ PIB/acre per application. As noted in Section 2.2, the average yield in the production of Gypchek is about 2×10^{9} PIBs per larva (Lewis 1971). Thus, at the lower application rate of $4 \cdot 10^{11}$ PIB/acre, the number of larval equivalents applied at the nominal application rate is about 200 larvae/acre [$4 \cdot 10^{11}$ PIB/acre $\div$ 2×10^{9} PIBs/larva]. At the higher application rate, the corresponding value is 500 larvae/acre [$1 \cdot 10^{12}$ PIB/acre $\div$ 2×10^{9} PIBs/larva]. This is actually a substantial overestimate because it does not consider the partial removal of insect parts during the production of Gypchek. By comparison, the density of gypsy moth larvae can be on the order of 10,000–100,000 larvae/acre. Thus, treatment during a severe infestation would increase exposure to the larvae by only about 0.2% [200 larvae/acre $\div$ 100,000 larvae/acre = 0.002] to 2%[200 larvae/acre $\div$ 10,000 larvae/acre = 0.02]. Treatment of areas

with a lower infestation rates would reduce exposure by inhibiting the increase in the larval population by a substantial amount with a subsequent reduction in LdNPV exposure.

3.2.3. Supplemental Extreme Exposures

While the approach taken by U.S. EPA (1996) is reasonable – i.e., provide no formal exposure assessment because no hazard is apparent – this risk assessment of LdNPV is part of a series of risk assessments involving several different control agents and at least a partial exposure assessment is developed in order to facilitate a comparison of risk among the different control agents that may be used by the Forest Service. For this risk assessment on Gypchek, the most plausible route of exposure for humans will involve the consumption of contaminated vegetation. While Gypchek is not used directly on food crops, it is plausible that home-grown vegetation could be incidentally contaminated in the aerial application of Gypchek.

As indicated in Section 2.3, Gypchek is applied at a rate of up to about 0.03 kg/acre – i.e., 30.6 g/acre for eradication – or about 0.066 lb/acre. The concentration of any material deposited on vegetation will depend on the characteristics of the vegetation (i.e., effective surface area to weight ratio) and application rate. In most Forest Service risk assessments (SERA 2001) as well as risk assessments conducted by U.S. EPA, empirical relationships proposed by Fletcher et al. (1994) are used to estimate initial concentrations on vegetation. For broadleaf forage plants, similar to those that might be grown in a domestic garden, Fletcher et al. (1994) estimate residue rates of 45 to 135 mg pesticide/kg vegetation per pound active ingredient applied. The consumption of homegrown vegetation is relatively well documented (U.S. EPA/ORD 1996). Individuals between the ages of 20 and 39 will typically consume about 0.000761 kg of homegrown vegetation per kg of body weight with 95% confidence intervals on consumption ranging from 0.0000777 to 0.00492 kg veg/kg bw (U.S. EPA/ORD 1996, Table 12-15, p. 9-14). Thus, taking the typical residue rate of 45 mg/kg vegetation and the typical consumption rate of 0.000761 kg veg/kg bw, the typical dose for an individual would be 0.034 mg Gypchek/kg bw. As an upper range on exposure, the 135 mg/kg residue rate may be used with the upper range on consumption, 0.00492 kg veg/kg bw, to calculate a dose of 0.66 mg Gypchek/kg bw.

A large number of other less extreme exposure scenarios could be developed for Gypchek but would serve little purpose in terms of assessing potential risk. As noted in Section 3.4, the upper range dose of 0.66 mg/kg bw is far below the no observed effect levels for Gypchek.

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview

Because no systemic toxic effects can be qualitatively identified for any plausible routes of exposure (i.e., oral, dermal, or inhalation), the U.S. EPA has not derived either an acute or chronic RfD for Gypchek. While this is a reasonable approach, the current risk assessment derives a surrogate acute RfD of 26 mg/kg bw based on an experimental acute NOAEL of 2,600 mg/kg bw in rats and the application of an uncertainty factor of 100. This approach is taken simply to provide a more quantitative basis for comparing the extremely low risks associated with the application of Gypchek to the risks posed by other agents that may be used to control the gypsy moth.

Technical grade Gypchek is an eye irritant. While this is not quantitatively considered in this risk assessment, the distinction between the irritant properties of technical grade Gypchek and the lack of eye irritation with Gypchek formulations as applied in the field is emphasized in order to highlight areas in which prudent handling practices are likely to be most important.

3.3.2. Surrogate RfD for Acute Exposures

The U.S. EPA (1996) did not propose a dose-response assessment for Gypchek or LdNPV. This approach is reasonable because no systemic toxic effects can be qualitatively identified for any plausible routes of exposure (i.e., oral, dermal, or inhalation). As noted in the exposure assessment, however, the current risk assessment on Gypchek is part of a series of risk assessments on several different agents. In order to facilitate an at least crude risk comparison among the different agents, a dose-response assessment for oral exposures will be developed.

As noted in Section 3.1.4, a single dose of LdNPV at 400 mg per rat was not associated with any adverse effects in male or female rats over a 30-day observation period (Terrell and Parke 1976a,b). At a somewhat higher dose, 500 mg per rat, a transient (2 week) but statistically significant decrease was noted in body weights over a 35-day observation period (Terrell et al. 1976c). For the purposes of this risk assessment, 400 mg will be taken as an acute NOAEL. Taking the upper range of the reported body weights of the rats – i.e., 150 grams or 0.15 kg – the 400 mg dose corresponds to a NOAEL of about 2,600 mg/kg bw. Following the general approach of a 10 fold-safety factor for sensitive subgroups and a 10 fold safety factor of for animal to human extrapolation, the 2,600 mg/kg bw dose will be divided by an uncertainty factor of 100 and a dose of 26 mg/kg bw will be adopted as a surrogate acute RfD for the risk characterization (Section 3.4).

3.3.3. Eye Irritation

Although Gypchek has a very low order of systemic toxicity, Gypchek may cause eye irritation and this endpoint is a concern at least for occupational exposures. This judgment is consistent with the assessment made by U.S. EPA (1996) in the re-registration of Gypchek. As discussed in Section 3.1.11, Gypchek is moderately irritating to the eyes when assayed at full strength (TGAI) in the rabbit eye (see discussion of Kuhn 1997a in Section 3.1.11). In the RED, the U.S. EPA (1996) noted the requirement for the following label warning concerning eye irritation for Gypchek:

> *a label statement is required indicating that these products are severe eye irritants and specifying appropriate eye protection. Toxicity Category I for primary eye irritation requires products containing the ais* [active ingredients] *to be labeled with the signal word "Danger" and the appropriate Statements of Precaution and Personal Protective Equipment, Practical Treatment, and Note to Physician.*

On review of the study using 2X Gypchek (Kuhn 1997b) in which no eye irritation was noted (Section 3.1.11), the U.S. EPA (Williams 1998) revised this assessment and concluded that:

> *The study [2X] demonstrated that the products, Gypchek and TM-Biocontrol, at concentrations twice standard dilution rate are "non-irritating".*

Thus, eye irritation may remain a concern in the manufacture or mixing of Gypchek and prudent industrial hygiene practices should be used to limit the possibility of contamination of the eyes.

3.4. RISK CHARACTERIZATION

3.4.1. Overview

There is no basis for asserting that any risk is plausible to either workers or members of the general public in the use of Gypchek to control the gypsy moth. 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 of about 50 to over 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 doses in the accidental exposure scenarios used to quantify risk.

3.4.2. Pathogenicity and Systemic Toxicity

Because Gypchek and LdNPV do not appear to cause adverse effects (Section 3.1), there is no basis for asserting that any risk is plausible to either workers or members of the general public in the use of Gypchek to control the gypsy moth. This conclusion is concurrent with the conclusions reach by U.S. EPA (1996) concerning the use of Gypchek as well as a related product, TM-Biocontrol:

> *The Agency does not expect any risk to humans or the environment from use of these biopesticides; therefore, all uses are eligible for reregistration. The bases of this decision are:*
>
> > *evaluation of the submitted data and published scientific literature for the RED indicate the data base is complete and acceptable for all data requirements;*
> >
> > *the fact that PIBs of OpNPV and LdNPV are naturally-occurring pathogens of gypsy moth and Douglas fir tussock moth and are selective for Lymantriids with no known adverse effects to any species other than the hosts, gypsy moth and Douglas fir tussock moth; and*
> >
> > *the fact that in approximately 20 years of use, there have been no reports of adverse human health and ecological effects, with the exception of possible dermal sensitivity and eye irritation in exposed humans during manufacture.*
>
> *–U.S. EPA, 1996, pp. 24-25*

In other words, there is no basis for asserting that any exposures to Gypchek are likely to harm either workers or members of the general public.

3.4.3. Extreme Exposure Scenarios

Notwithstanding the above assertions, this risk assessment does attempt to quantify risk from one extreme exposure scenario – the inadvertent spray of a home garden. This is an extreme scenario because Gypchek should not be applied to any vegetation other than tree species that contain gypsy moth larvae (U.S. EPA 1996). Nonetheless, in aerial applications, an accidental spray of a home garden could occur. Based on the upper range of the application rate, the upper range of contamination rates, and the upper range of the consumption of homegrown vegetation, the highest estimated dose is 0.66 mg/kg bw (Section 3.2.3). Based on the surrogate acute RfD of 26

mg/kg bw (Section 3.3.2), this results in a hazard quotient of 0.02, below the level of concern (i.e., a hazard quotient of one) by a factor of 50. Other more plausible exposure scenarios would lead to much smaller hazard quotients. For example, based on the upper range of the application rate but using the typical residue rate typical consumption rate, the typical dose for an individual would be 0.034 mg Gypchek/kg bw, with a corresponding hazard quotient of 0.0013, which is below the level of concern by a factor of over 750.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview.

Similar to the hazard identification for the human health risk assessment, there is no indication that LdNPV or the Gypchek formulation of LdNPV has the potential to cause any 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 of available studies in mammals, few studies are available in birds but the results of these studies are essentially identical to those in mammals indicating that exposures to LdNPV at levels that are substantially higher than those likely to occur in the environment will not be associated with any adverse effects. Based on bioassays of LdNPV on the large number of nontarget insect species and supported by the general high species specificity of related baculoviruses, the hazard identification for LdNPV in nontarget insects is essentially identical to that in birds and mammals. There is no indication that adverse effects will be caused in nontarget insects at any level of exposure. Relatively few studies have been conducted in fish and aquatic invertebrates but these studies are consistent with studies in terrestrial species and indicate that effects on fish or aquatic invertebrates are unlikely. 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.

4.1.2. Toxicity to Terrestrial Organisms.

4.1.2.1. Mammals – The hazard identification for mammals is closely related to the hazard identification for the human health risk assessment (Section 3.1) in that both may be based, at least partially, on a number of standard toxicity studies in experimental mammals (Appendix 1). As summarized in Appendix 1 and discussed in Section 3.1, adverse systemic effects caused by Gypchek or LdNPV have not been observed in mammals. Except for eye irritation, there is little indication that LdNPV or the Gypchek formulation of LdNPV will have any effect in mammals even at extremely high levels of the exposure. The relationship of plausible exposures to any potential effect is discussed further in Section 4.4 (Risk Characterization).

One study has been specifically conducted on wildlife mammals – i.e., mammals other than the common test species used in the human health risk assessment. As summarized in Appendix 1, Lautenschlager et al. (1977) exposed mice, short-tailed shrews, and opossums to various forms of LdNPV: gypsy moth larvae infected with LdNPV, a purified formulation of LdNPV, and a spray preparation of LdNPV. Based on both gross observations as well as necropsy and microscopic examination of several different tissues, no effects were seen in any species. Again, this is consistent with the relatively complete set of standard toxicity studies available on commonly used laboratory mammals (Section 3.1). In addition, as discussed in Section 3.1.9, reproduction in paired mice was higher in the LdNPV treated mice than the control group. While this study was not a formal or standard assay for reproductive performance, it is the only reproduction study available. Consistent with the other toxicity studies on LdNPV, the results provide no basis for asserting any plausible hazard in mammals exposed to LdNPV or the Gypchek formulation.

4.1.2.2. Birds – The available studies in birds are detailed in Appendix 2. Relative to the large number available studies in mammals, few studies are available in birds but the results of these studies are essentially identical to those in mammals indicating that exposures to LdNPV at levels that are substantially higher than those likely to occur in the environment will not be associated with any adverse effects.

One relatively standard dietary exposure study has been conducted in mallard ducks, a common test species for assessing the effects of pesticides on birds (Roberts and Wineholt 1976). At exposure levels of up to 1.04×10^9 PIBs/g of feed (estimated by the authors to represent exposures equivalent to 100 times the normal application rate), no adverse effects associated with treatment were observed. As with most toxicity studies in birds, clinical biochemistry and histopathology were not conducted.

In a field simulation study (Podgwaite and Galipeau 1978), black-capped chickadees and house sparrows were fed LdNPV infected gypsy moth larvae every other day for 3 weeks. This study included histopathology and, as with the comparable studies in mammals, no adverse effects were noted based on histopathology, changes in body weight or gross signs of toxicity.

Lautenschlager et al. (1976b) conducted a field study on resident songbirds and caged quail in areas treated with two different formulations of LdNPV (see Appendix 2 for details). Consistent with the standard toxicity studies, no evidence of direct adverse effects from exposure to LdNPV were noted. In addition, the study noted no secondary adverse effects on birds that use gypsy moth larvae as a food source. Compared to untreated plots that were infested with gypsy moth larvae, the secondary effect of LdNPV treatments appeared to be an enhancement songbird habitat secondary to a reduction in defoliation from gypsy moth larvae.

4.1.2.3. Terrestrial Invertebrates – The primary characteristic of LdNPV as well as many related viruses involves a very high degree of host specificity – i.e., the virus is pathogenic to one or only a very small number of species. LdNPV specifically is a member of the Baculoviridae that includes both nucleopolyhedroviruses, such as LdNPV and OpNPV, as well as granuloviruses (Döller 1985). Both budded viruses and occluded viruses are produced by baculoviruses. The budded viruses participate in cell to cell spreading of the infection, and the occluded viruses participate in the spread of the infection among individual insects in a population (Russell and Rohrmann 1997, Theilmann et al. 1996). Baculoviruses have been isolated only from arthropods and are characterized by a very limited host range (Chou et al. 1996).

This general tendency for host specificity in baculoviruses has been demonstrated for LdNPV. As summarized in Appendix 3, LdNPV has been assayed in 46 species of nontarget Lepidoptera (Barber et al. 1993), 17 genera and 31 species of ants (Wang et al. 2000), as well as a species of fly (Barber et al. 1993), the common honey bee (Cantwell et al. 1972; Knoz 1970), and the leafcutting bee (Barber et al. 1993). The studies by Barber et al. (1993) specifically assayed for infectivity and found no indication that LdNPV is pathogenic to any insect species except the gypsy moth. No adverse effects were observed in any species tested in any of these studies. In addition, the recent field study by Rastall et al. (2003) noted no effects in nontarget insects after the application of Gypchek. In this study, Gypchek was applied at a rate of 2×10^{11} OB/acre in May of 1997 and 1998 to two forests susceptible to gypsy moth. Nontarget lepidoptera were monitored in two pre-treatment year as well as in treatment years. No statistically significant effects were associated with the Gypchek applications.

Thus, based on the large number of species assays with LdNPV, a recent field study, and supported by the general high species specificity of related baculoviruses, the hazard identification for nontarget insects is essentially identical to that in birds and mammals. There is no indication that adverse effects will be caused in nontarget insects at any level of exposure.

4.1.2.4. Terrestrial Plants (Macrophytes) – No phytotoxicity studies on LdNPV were encountered and the U.S. EPA waived the requirement for such tests (U.S. EPA 1996). This appears to be a reasonable approach in that there is no basis for supposing that LdNPV is likely to be toxic to any form of vegetation. The only effect that is plausible is the protective effect that LdNPV will have in terms of preventing damage to vegetation from gypsy moth larvae.

4.1.2.5. Terrestrial Microorganisms – No studies have been encountered on the effects of LdNPV on terrestrial microorganisms. There is no apparent basis for asserting that direct effects – i.e., microbial toxicity – are plausible. The protective effect of LdNPV on vegetation is likely to affect soil microorganisms in that the microbial soil community is likely to change secondary to changes in terrestrial vegetation.

4.1.3. Aquatic Organisms.

4.1.3.1. Fish – Two studies are available on the toxicity of LdNPV to fish (Moore 1977; Kreutzweiser et al. 1997) and the results of both studies are consistent with the data on terrestrial species: there is no indication of toxicity or pathogenicity.

In the study by Moore (1977), a "crude nuclear-polyhedrosis virus preparation" was tested in both bluegill sunfish and brown trout. Fish were exposed to LdNPV for 96 hours and observed for 30 days after exposure. The test concentrations are given in the study as 7.5×10^8 PIB/gram of fish or 1.5×10^9 PIB/gram of fish (Moore 1977, Table 2, p. 10). Details on how these exposures are calculated are not given. In addition to standard observations for mortality, appearance and general behavior, histopathology was conducted on gill arches, stomach, liver, and intestines. Fish were equally divided among control groups, low concentration and high concentration groups. A total of 240 fish of each species were used and no treatment related effects were noted in either species.

Kreutzweiser et al. (1997) assayed LdNPV in rainbow trout after the viruses were fed to the trout in standard feed pellets at a dose of 1.6×10^6 occlusion bodies (OBs)/fish. Since each fish weighed approximately 6 g, this corresponds to a dose of about 2.7×10^8 OBs/kg bw. The study covered a 21-day treatment period in which the fish were fed on days 1, 3, 5, 8, 10, 12, 15, 17, and 19. No effects were noted on mortality, behavior, growth rate, or gross pathological examination of the internal organs. In addition, no viable NPV was detected in the stomach or intestinal tract. As reviewed by Kreutzweiser et al. (1997), these results are consistent with the general observation that *"NPVs cannot induce protein production nor reproduce in vertebrate cells in general"*. (Kreutzweiser et al. 1997, p. 68, column 1).

4.1.3.2. Amphibians – No data have been encountered on the effects of NPV exposures to amphibians.

4.1.3.3. Aquatic Invertebrates – Only one study (Streams 1976) has been encountered on the toxicity of LdNPV to aquatic invertebrates. This study, however, involved five species: *Daphnia magna* (a commonly used test species in aquatic toxicology), backswimmers (*Notonecta undulata*), midge larvae (*Chironomus thummi*), and two species of water boatmen (adult *Hesperocorixa interrupta* and *Sigara gordita*). As detailed in Appendix 4, no effects were observed on mortality or reproduction in any species over exposure periods of up to four weeks.

While this study is not a standard bioassay typically conducted on pesticides, it provides much more detailed information than standard bioassays and has been accepted by U.S. EPA (1996) as indicating no apparent toxicity to aquatic invertebrates.

4.1.3.4. Aquatic Plants – As with terrestrial plants, no studies have been conducted on the toxicity of LdNPV to aquatic plants. Given the lack of any biological basis for asserting that direct effects on aquatic plants are plausible, this does not add substantial uncertainty to the risk assessment. The U.S. EPA (1996) has explicitly waived the requirements for toxicity testing in nontarget plant species.

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview

In ground or aerial applications, it is likely that a large number of species could be exposed to Gychek/LdNPV. Because of the apparently very low toxicity of Gypchek and LdNPV, the need for any formal exposure assessment is questionable. Nonetheless, in an attempt to provide some bases for comparing the potential risks of Gypchek to 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 mg Gypchek /kg bw to 3.2 mg Gypchek /kg bw. For aquatic organisms, concentrations are expressed in units of PIB/liter 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. A large number of other less extreme exposure assessments could be developed but these would not alter the assessment of risk since these extreme exposure assessments are substantially below any level of concern.

4.2.2. LdNPV and Gypsy Moth Parts in Gypchek

As with the human health risk assessment, a formal exposure assessment for Gypchek is not necessary because of the failure to identify any adverse effects. As discussed in section 3.2, the application of Gypchek in areas infested by the gypsy moth will not substantially increase exposure to either LdNPV or the larval parts (e.g., hairs) that contaminate Gypchek. To the contrary, treatment of gypsy moth infestations with Gypchek is likely to reduce longer term exposures to both the larval parts and the virus by reducing the population of gypsy moth and lessening the chance of a substantial increase in the gypsy moth population (U.S. EPA 1996).

4.2.3. Supplemental Extreme Exposures

As with the human health risk assessment (Section 3.2), some extreme exposure scenarios will be developed for Gypchek and used in the risk characterization (Section 4.4). Again, this approach is taken to facilitate comparisons of risk among the various agents that may be used to control or eradicate gypsy moth infestations. Two specific exposure scenarios are developed: one for a large vertebrate consuming vegetation directly sprayed with Gypchek and the other for aquatic species in a small pond directly sprayed with Gypchek. Both of these scenarios should be regarded as extreme, since efforts are made in the application of Gypchek to avoid contamination of vegetation that will not be habitat for the gypsy moth (e.g., grasses) as well as incidental contamination of open water.

4.2.3.1. Contaminated Vegetation – For terrestrial species, an exposure assessment is developed for a large herbivore, such as a deer, consuming contaminated vegetation. The general approach is similar to that used in the human health risk assessment except that the deer is assumed to consume contaminated grass rather than broadleaf vegetables. This approach is taken because contaminated grass is estimated to have higher residue rates – i.e., 85 and 240 mg pesticide/kg vegetation per pound active ingredient applied per acre – than the corresponding values for broadleaf vegetation – i.e., 45 mg pesticide/kg vegetation to 135 mg pesticide/kg vegetation per pound active ingredient applied per acre (Fletcher et al. 1994). Thus, at an application rate of 0.066 lb Gypchek/acre (Section 2.3), the estimated initial residues on vegetation would be in the range of about 5.6 mg Gypchek/kg vegetation [85 mg pesticide/kg vegetation per lb/acre × 0.066 lb/acre = 5.61 mg/kg] to 16 mg Gypchek/kg vegetation [240 mg pesticide/kg vegetation per lb/acre × 0.066 lb/acre = 15.84 mg/kg].

In order to estimate the dose to the deer, the amount of vegetation consumed must be estimated. This will be highly variable, depending on the amount of grass consumed relative to other types

of vegetation and the amount of time spent grazing at the treated site. As a very conservative upper limit, it will be assumed that the deer consumes its caloric requirement for food totally as contaminated grass. Caloric requirements for mammals are well-characterized. The U.S. EPA/ORD (1993, p. 3-6), recommends the following relationship based on body weight (BW): $kcal/day = 1.518 \times W(g)^{0.73}$. Based on this relationship, a 70 kg deer would require approximately 5226 kcal/day [$1.518 \times 70{,}000\ g^{0.73} = 5226.288$]. The caloric content of vegetation is given by U.S. EPA/ORD (1993, p. 3-5) as 2.46 kcal/gram vegetation dry weight with a corresponding water content of 85% (U.S. EPA/ORD 1993, p. 4-14). Correcting the dry weight caloric content to wet weight, the caloric content of the grass will be taken as 0.369 kcal/g [2.46 kcal/gram vegetation dry weight × (1-0.85) = 0.369 kcal/g]. Thus, the 70 kg deer would consume about 14.2 kg of grass per day [5226 kcal/day ÷ 0.369 kcal/g = 14,162.6 g, which is equal to about 14.2 kg].

At the lower range of the estimated residue rate of 5.6 mg Gypchek/kg vegetation, the estimated dose to the deer would be 1.1 mg Gypchek /kg bw [5.6 mg Gypchek/kg vegetation × 14.2 kg vegetation ÷ 70 kg bw = 1.136 mg Gypchek /kg bw]. At the upper range of the estimated residue rate of 16 mg Gypchek/kg vegetation, the estimated dose to the deer would be about 3.2 mg Gypchek /kg bw [16 mg Gypchek/kg vegetation × 14.2 kg vegetation ÷ 70 kg bw = 3.2457 mg/kg bw].

4.2.3.2. Small Pond – For the risk characterization of aquatic species, one extreme exposure scenario is developed in which a small pond is directly sprayed with Gypchek at the highest application rate. As discussed in Section 4.3.3, the toxicity data for aquatic species is expressed in units of PIB/L. The highest application rate for Gypchek is 1×10^{12} PIB/acre (Section 2.3).

For this exposure scenario, the small pond will be characterized as 1000 m^2 in surface area with an average depth of 1 meter. An application rate of 1×10^{12} PIB/acre corresponds to about 2.5×10^{8} PIB/m^2 [1×10^{12} PIB/acre ÷ 4047 m^2/1 acre = 2.471×10^{8} PIB/m^2]. At a depth of 1 meter, each square meter of pond surface would correspond to 1 cubic meter of water or 1,000 liters. Thus, assuming instantaneous mixing, the concentration in the water would be 2.5×10^{5} PIB/L [2.5×10^{8} PIB ÷ 1000 L]. This concentration will be used directly to characterize risks to aquatic species.

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Overview

Because no hazards can be identified for any species, a quantitative dose-response assessment is not required and no such assessments have been proposed by U.S. EPA and no quantitative dose-response assessments were used in the previous USDA risk assessment for Gypchek. In order to provide a clear comparison of the risks of using Gypchek relative to other agents, dose-response assessments are proposed in the current risk assessment for both terrestrial mammals and aquatic species. For terrestrial mammals, the NOAEL of 2,600 mg/kg bw is used. This is the same NOAEL that served as the basis for the surrogate acute RfD in the human health risk assessment. For aquatic species, only NOEC values are available and the highest NOEC of 8×10^{9} PIB/L is used to characterize risk.

4.3.2. Qualitative Assessment

There is no basis for asserting that Gypchek poses any risk to nontarget species. Consequently, a standard dose-response assessment is not required for any species or groups of species and the previous USDA (1995) risk assessment does not propose a quantitative dose-response assessment for any wildlife species. This is essentially identical to the approach and conclusions reached by U.S. EPA (1996) in the re-registration eligibility decision for both Gypchek and TM-Biocontrol:

> *The available avian and aquatic data and other relevant literature and information show that PIBs of OpNPV and LdNPV do not cause adverse effects on avian, mammalian and aquatic wildlife. No mortalities were seen when these viruses were fed to mallard ducks, house sparrows, bobwhite quail and black-capped chickadees. No mortalities or other adverse effects were seen in brown trout, bluegill sunfish, and a variety of aquatic invertebrates. Similarly, tests with mule deer, Virginia opossums, short-tailed shrews and white-footed mice, resulted in no evidence of pathogenicity or toxicity. Known insect host range and scientific literature on honey bee mortality demonstrate that these baculoviruses do not have adverse effects on honeybees and should not pose a significant risk to nontarget insects (Cantwell et al. 1972; Knox 1970). NPV effects on endangered species are considered a low risk based on the absence of threat to nontarget organisms.* (U.S. EPA 1996, pp. 23-24)

4.3.3. Quantitative Assessments

While the qualitative approach to assessing the potential effects in nontarget species is clearly justified, the current risk assessment quantifies extreme exposures to Gypchek for both a terrestrial herbivore and aquatic species (Section 4.2.3). As in the human health risk assessment, this approach is taken to permit a clearer comparison of risks among the different agent that may be used in response to gypsy moth infestations.

For a large herbivore consuming vegetation, exposures are expressed in units of mg Gypchek/kg vegetation and the NOAEL of 2,600 mg Gypchek/kg bw used as the basis for the surrogate acute RfD (Section 3.3.2) can used to characterize risks for the large herbivore. As discussed in Section 3.3.2, this NOAEL of 2,600 mg Gypchek/kg bw is based on the study by (Terrell and Parke 1976a,b) in which rats weighing 100 to 150 grams were dosed with 400 mg Gypchek and no adverse effects were noted over a 30-day observation period. At a somewhat higher dose, 500 mg Gypchek/rat, decreased food consumption with a corresponding decrease in body weight was observed in a study by the same investigators (Terrell et al. 1976c). These studies are detailed further in Appendix 1.

As discussed in Section 4.1.3, there are no studies indicating that Gypchek will be toxic or pathogenic to any aquatic organisms under any exposure conditions. The most recent study, Kreutzweiser et al. (1997), involved feeding trout with contaminated food pellets. While this study is useful for the qualitative assessment of pathogenicity and toxicity, the route of exposure is not suitable for use in a quantitative risk assessment.

The other two studies that could be used both involved exposures to Gypchek in water. The study in invertebrates (Streams 1976) used concentrations of 250 polyhedra/mL or 2.5×10^5 PIB/L. The study in fish (Moore 1977) expresses exposures in units of PIB/gram of fish (Section 4.1.3.1). Moore (1977) does not specifically convert the exposure units in PIB/g fish to more typical concentrations (e.g., PIB/liter of water) but does indicate loadings in units of grams of fish per liter of water. For bluegills, the loading factor was 0.23 grams of fish per liter of water. Thus, the concentrations would correspond to approximately 1.7×10^8 PIB/liter [7.5×10^8 PIB/gram of fish × 0.23 grams fish/L = 1.725×10^8 PIB/liter] and 3.45×10^8 PIB/liter [1.5×10^9 PIB/gram of fish × 0.23 grams fish/L = 0.345×10^9 PIB/liter]. For trout, the loading factors were 5.31 grams of fish per liter of water and the corresponding concentrations were about 4×10^9 PIB/liter [7.5×10^8 PIB/gram of fish × 5.31 grams fish/L = 39.825×10^8 PIB/liter] and 8×10^9 PIB/liter [1.5×10^9 PIB/gram of fish × 5.31 grams fish/L = 7.965×10^9 PIB/liter].

All of these exposures are essentially NOEC's values – i.e., no effects were observed at any concentrations. In the absence of an LOEC, the most appropriate value to use in risk characterization is the highest NOEC, in this case 8×10^9 PIB/liter from trout in the study by Moore (1977). In other words, if a large number of NOEC values are available with no indication that any concentration will cause an adverse effect, it is appropriate and conservative to use the highest NOEC because this NOEC is still below any concentration that would be anticipated to cause an adverse effect. While the use of the lowest NOEC would be "more conservative", it would tend to distort rather than clarify risk.

4.4. RISK CHARACTERIZATION

4.4.1. Overview

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 appear to be pathogenic and toxic to the gypsy moth and only to the gypsy moth.

Because Gypchek does not appear to cause adverse effects, quantitative expressions of risk are in some respects more difficult because clear NOEC and LOEC values cannot be defined – i.e., if an agent is not shown to cause an effect, the threshold exposure level 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.

4.4.2. Qualitative Assessment

Gypchek does not appear to be capable of causing adverse effects in any species other than the gypsy moth. Thus, the use of Gypchek to control or eradicate gypsy moth infestations appears to carry no identifiable risk. This is essentially identical to the conclusions reached by U.S. EPA (1996) in the re-registration of LdNPV and OpNPV:

> *Due to the lack of adverse effects on avian, mammalian and aquatic wildlife, plants and nontarget insects documented in the submitted studies and scientific literature after 20 years of use, the Agency finds that the PIBs of L. dispar and O. pseudotsugata NPVs pose minimal or no risk to nontarget wildlife, including endangered species.*

The current re-evaluation of the available information supports this basic conclusion with no reservations.

As in the human health risk assessment, there are basically two agents that could be of concern in the use of Gypchek: the virus and the insect parts. As discussed in Section 3.1 and 4.1, there is no indication that LdNPV is pathogenic or otherwise toxic to any species other than the gypsy moth. To the contrary, experience with this as well as other related NPVs indicate that these viruses have a very narrow host range. As is also true for the human health risk assessment, the overriding consideration in the risk characterization for nontarget species is that the use of Gypchek will decrease rather than increase exposure to the gypsy moth and LdNPV (Section 3.2.2).

4.4.3. Quantitative Assessments

The above qualitative assessment is adequate for assessing the plausibility of intended harm from the use of Gypchek to control or eradicate gypsy moth populations. This risk assessment, however, is part of a larger effort to review the risks associated with the use of several different and diverse agents and some quantitative expression of risk for Gypchek is useful both in further demonstrating the apparent safety of this agent and in comparing potential risks among the different agents that may be used.

Based on the exposure assessment (Section 4.2) and dose-response assessment (Section 4.3), two such expressions of risk may be made: one for a large mammal consuming contaminated vegetation and the other for aquatic species in a small pond directly sprayed with Gypchek. As

detailed in Section 4.2.3.1, a large mammal grazing exclusively on grass directly sprayed with Gypchek at the highest application rate might consume as much as 3.2 mg Gycheck/kg body weight. Using the acute NOAEL of 2,600 mg Gypchek/kg bw (Section 4.3.3), this exposure would correspond to a hazard quotient of 0.001 [3.2 mg Gycheck/kg body weight ÷2,600 mg Gypchek/kg bw = 0.00123]. In other words, the maximum level of exposure is below the NOAEL by a factor of about 1000. This numeric expression of risk is thus consistent with the qualitative risk characterization offered by U.S. EPA (1996) and the previous risk assessment on Gypchek (USDA 1995).

For aquatic species, the direct spray of a small pond is estimated to result in initial concentrations of about 2.5×10^5 PIB/L. This is a reasonable worst case scenario in that direct spray of the pond at the highest application rate is assumed. Because there is no indication that any concentration of Gypchek will cause any effect in any aquatic species, the highest available NOEC is used to characterize risk – i.e., 8×10^9 PIB/liter from the trout study by Moore (1977), as discussed in Section 4.3.3. Thus, the hazard quotient is 0.00003 [2.5×10^5 PIB/L ÷ 8×10^9 PIB/liter = 0.00003125], as factor of over 30,000 below the NOEC. Again, this numeric expression of risk is in agreement with the qualitative conclusions reached by U.S. EPA (1996) and USDA (1995).

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Note: *Designations such as 19??c are used by U.S. EPA to identify submissions whose date is unclear. This designation is also used below for consistency with U.S. EPA.*

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APPENDICES

Appendix 1: Toxicity of LdNPV in Mammals

Appendix 2: Toxicity of LdNPV in Birds

Appendix 3: Toxicity of Gypsy Moth LdNPV in Nontarget Insects

Appendix 4: Toxicity of Gypsy Moth NPV in Aquatic Invertebrates

NOTE: Several of the studies summarized in these appendices appear to have been submitted to U.S. EPA on more than one occasion and some with an inconsistent list of authors. This is indicated in the appendices by multiple references given for the same data summary. Unless otherwise specified, the multiple cited references for the same data are identical study submissions. The multiple references are maintained in the appendices simply to avoid confusion that might be associated with "missing" MRID numbers.

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

Product	Species/Exposure	Observations	Reference
ACUTE ORAL			
Gypsy Moth NPV prepared as 20% suspension in distilled water	Single oral dose of 400 mg test material to 20 male and 20 female Sprague Dawley rats. Negative control group consisted of 20 males and 20 females. All rats were observed for 30 days. Animals weighted between 100 and 150 grams.	No mortality and no adverse effects on behavior throughout the 30-day observation period. No treatment-related gross pathological findings. NOTE: Although this is called a "feeding study" the precise route of exposure is not specified.	Terrell and Parke 1976b MRID 00048862 Terrell and Parke 1976a MRID 00055915
Gypsy Moth NPV prepared as 20% suspension in distilled water	Single oral dose of 500 mg test material to 20 male and 20 female Sprague Dawley rats. Negative control group consisted of 20 males and 20 females. All rats were observed for 35 days. Animals weighted between 100 and 150 grams.	Mortality in 8 control animals and 3 treated animals, all of which exhibited overt physical and or behavioral changes including piloerection, decreased locomotor activity, increased respiratory rate, and decreased body weight. Adverse treatment-related effects included statistically significant decreases in body weights of males for the first 2 weeks and statistically significant decreases in food consumption for males and females during the first week. No treatment-related adverse effects were noted regarding body temperature, hematological and clinical chemistry results, urinalysis parameters or necropsy examinations.	Terrell et al. 1976c MRID 00048863
L. dispar NPV (Lot 33)	Single oral gavage dose of NPV suspended in 0.9% saline at a concentration of 0.2 g/mL (equivalent to 1.32 PIB/mL) administered to fasted young adult rats (30 males and 30 females, weighing approximately 125 g). Rats were observed daily for 30 days.	No signs of toxicity observed; no mortality.	Hart 1976 MRID 00068401

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

Product	Species/Exposure	Observations	Reference
ACUTE ORAL (continued)			
P. dispar[1] NPV	Single oral gavage dose of test compound in 0.8% saline at a concentration of $40x10^9$ polyhedra/mL (dosage was 1 mL of the stated suspension per rat) to 20 male and 20 female Sprague Dawley weanling albino rats. Negative controls (20 males and 20 females) received saline	No mortality and no overt signs of toxicity during the 35-day observation period.	Hart and Thornett 1975c MRID 00049263 Hart et al.1975a MRID 00060702 [Final Report]
P. dispar[1] NPV intact polyhedra (suspensions contained $1.8x10^{11}$ polyhedra/g)	Single virus exposure (gastric intubation) to 0.02 g/animal polyhedra to adult mice [10 males (5 untreated and 5 immunosuppressed) and 10 females (5 untreated and 5 immunosuppressed)]. Immunosuppressed mice were *selectively depleted of cell-mediated immune function by thymectomy and treatment with anit-lymphoctye serum* (cytoxan administered ip at 300 mg/kg/mouse). Positive controls treated with *autoclaved* polyhedra; negative controls treated with saline. All animals observed for 21 days.	No treatment related adverse effects observed; no mortality among immunosuppressed mice; no lesions noted grossly post-mortem. Serological results indicated that the animals with intact immune systems were exposed to NPV antigen, since positive reactions were apparent with autoclaved and non-autoclaved NPV preparations. Control (saline) exposure did not produce antibody responses.	Shope et al. 1975 MRID 000606700

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

Product	Species/Exposure	Observations	Reference
LONGER TERM ORAL			
NPV of the gypsy moth	Mammalian predators of the gypsy moth (40 white-footed mice caged in pairs; 6 short-tailed shrews caged individually; and 2 Virginia opossums caged individually) were collected in the field and exposed orally to NPV in the form of NPV-infected 5th gypsy moth larvae, PIBs mixed in dog food, and PIBs mixed in a standard spray formulation for 20 days. All animals were sacrificed on day 32. *The total amount of NPV consumed by each test mouse and shrew was equivalent to more than a 40-ha exposure for a 70 kg person assuming that NPV was applied at the rate of 5.0x10^{11} PIB/ha*. No further details regarding these estimates are provided.	No adverse effects were observed related to general body condition, weight, or reproductive efficiency (mice only species tested). In addition, necropsy and microscopic examination revealed no abnormalities resulting from exposure to NPV.	Lautenschlager et al. 1977 MRID 00134314
NPV of the Gypsy Moth in distilled water	Administration of daily doses of 0, 10^7, 10^8, or 10^9 PIBs/animal to young adult, purebred beagles (13 males and 14 females) over a period of 90 days. These doses correspond Gypchek doses of 0, 1.8, 18, and 180 mg/dog or approximately 0.2, 1.6, and 17 mg/kg/day based on terminal body weights in each dose group. The doses were delivered directly into the mouth of each dog and small amounts of sugar were added just before dosing to increase palatability.	No evidence of toxicity. All treated and control animals were in good health throughout the study. Standard hematology, clinical biochemistry, and urinalysis were conducted on each animal at or before the start of exposure and at 2, 4, and 6 months after the start of exposure. After sacrifice, standard examinations were conducted for signs of gross pathology or histopathology. No treatment related effects were observed.	Hart and Wosu 1975 MRID 00060698 Hart 1975a MRID 00067103 [Final Report]

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

Product	Species/Exposure	Observations	Reference
LONGER TERM ORAL (continued)			
P. dispar$_1$ NPV	Sprague Dawley rats (50 males and 50 females/dose group) exposed to dietary concentrations of 0, 10^7 or 10^8 PIB/rat/day for 2 years. These doses correspond to Gypchek daily doses of 1.8 or 18 mg/rat. The average terminal body weights (both sexes combined) was approximately 400 g. Thus, the approximate average dose rate was 4.5 or 45 mg Gypchek/kg body weight.	Observations included body weight, food consumption, gross signs of toxicity, and pathology. No treatment-related effects on survival and no significant differences in tumor incidence or other lesions in treated rats, compared with controls. Authors indicate *overall survival to termination at 104 weeks was 137/299 or 46%. Individual groups ranged from 32 to 60% with both extremes falling in the high dosage group. It seems clear that treatment did not influence survival.*	Hart 1975b MRID 00049267 Hart and Cockrell 1975 MRID 00060699
DERMAL			
P. dispar[1] NPV	Dermal application of 1/10 of 1 mL of test compound in 0.8% saline at a concentration of $40x10^9$ polyhedra/mL or freed virus rods prepared from dry polyhedra to shaved and abraded or shaved and intact skin of albino guinea pigs (5 males and 5 females/dose group). Treated sites were covered by 1"x1" gauze pads held in place by tape and covered by impermeable binding (rubber dam) for 24 hours. Animals were observed for 21 days after treatment.	No mortality and no evidence of irritation (either erythema or edema) resulting from exposure to NPV of the Gypsy Moth either as the polyhedra themselves or as virus rods freed from the polyhedra throughout observation period. No evidence of systemic toxicity.	Hart and Thornett 1975d MRID 00049263 Hart et al. 1975b MRID 00060703 [Final Report]

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

Product	Species/Exposure	Observations	Reference
DERMAL ***(continued)***			
P. dispar[1] NPV	Dermal application of 0.5 mL test material (*P. dispar*[1] NPV suspended in 0.8% saline at the rate of $40x10^9$ polyhedra/animal) to shaved and abraded skin (3 rabbits) or shaved and intact skin (3 rabbits). Treated sites were covered with 1" sq gauze patch and held in place with adhesive tape. Entire trunks were wrapped with nonabsorbent binder for 24 hours. After 24-hour exposure, the skin was cleaned and the reactions were scored immediately and again at 72 hours after exposure.	Primary irritation score = 0; there was no evidence of irritation in either intact or abraded skin and no edema was observed. Body temperatures were within normal temperature range except in one rabbit whose temperature was slightly depressed at 24, 48, and 72 hours. This finding is judged to be idiosyncratic and not significant.	Hart and Thornett 1975b MRID 00066104
P. dispar[1] NPV intact polyhedra	Dermal application of 0.04 g saline (negative controls), autoclaved polyhedra (positive controls) or polyhedra to shaved backs of 5 male and 5 female albino guinea pigs with depressed cell-mediated immune functions after cortisone treatment (300 mg/kg ip)on two areas of intact skin and one ear. Exposed ears were measured for 7-10 days; areas larger than 16mm were considered positive.	NPV treatment to ears caused positive responses in 3/5 males and 5/5 females without immunosuppressive treatment. In animals with depressed cell-mediated immune functions due to cortisone treatment, NPV caused positive responses in 3/5 males and 2/5 females. None of the immunosuppressed animals died during the observation period.	Shope et al. 1975 MRID 000606700 Shope et al. 1977
P. dispar[1] NPV	Dermal application of $40x10^9$ polyhedra suspended in 0.8% saline (dose = 0.5 mL) to shaved abraded or intact skin of New Zealand white rabbits (3/dose group) occluded for 24 hours. Skin cleaned after 24-hour exposure and observed at 24 and 72 hours.	No irritation or edema at 24 or 72 hours after exposure on abraded or intact skin. Primary skin irritation score is zero.	Hart and Thornett 1975e MRID 00049265
L. dispar NPV (Bioserv Lot 33)	Dermal application of 1 g/animal to abraded and intact skin on approximately 10% of the body surface of New Zealand white rabbits (2 males and 2 females/dose group). Daily observations were made for 21 days after treatment.	No mortality. Test compound did not cause dermal toxicity or abnormal behavior in any of the animals throughout the 21-day observation period. No treatment-related gross pathological or histopathological effects were observed.	Becker and Parke 1976b MRID 00060694 Becker et al. 1976 MRID 00066101

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

Product	Species/Exposure	Observations	Reference
OCULAR			
P. dispar[1] NPV intact polyhedra	Single virus exposure (eye irritation study, NOS) to 0.01 g/animal polyhedra to adult mice [10 males (5 untreated and 5 immunosuppressed) and 10 females (5 untreated and 5 immunosuppressed)]. Positive controls treated with *autoclaved* polyhedra; negative controls treated with saline. All animals observed for 21 days.	Immunosuppressed mice were selectively depleted of cell-mediated immune function by thymectomy and treatment with anti-lymphocyte serum (cytoxan administered i.p. at 300 mg/kg/mouse). No eye irritation noted.	Shope et al. 1975 MRID 000606700
P. dispar[1] NPV	Administration of test compound in 0.8% saline at a rate of $40x10^9$ polyhedra per animal to the left eye (conjunctival sac) (dose = 0.1 mL per animal) of 5 male and 5 female New Zealand white rabbits. Right eye served as control and received 0.1 mL of 0.8% saline. Animals examined for injury at 24, 48, and 72 hours.	No significant signs of irritation.	Hart and Thornett 1975a MRID 00049264 Hart and Thronett 1975f MRID 00060704 [Final Report]

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

Product	Species/Exposure	Observations	Reference
OCULAR *(continued)*			
P. dispar[1] NPV	Administration of freed virus rods at a concentration corresponding to $40x10^9$ polyhedra/mL of 0.8% saline to the left eye (conjunctival sac) (dose = 0.1 mL per animal) of 5 male and 5 female New Zealand white rabbits. Right eye served as control and received 0.1 mL of 0.8% saline. Animals examined for injury at 24, 48, and 72 hours.	No significant signs of irritation.	Hart and Thornett 1975a MRID 00049264 Hart and Thronett 1975f MRID 00060704 [Final Report]
"Gypsy Moth Virus" ($6.48x10^{10}$/g) (Lot 35) described as light grey powder	Administration of 50 mg of test compound in to one eye of each of 9 male New Zealand white (albino) rabbits, other eye of each rabbit served as control. After administration, treated eyes of 3 rabbits were washed with 20 mL of lukewarm dionized water 1 minute after treatment. The eyes of 3 other rabbits were washed 5 minutes after treatment and the eyes of the remaining 3 rabbits were not washed after treatment.	One rabbit from the 1-minute wash died after 1 day, but the death was not considered to be treatment related. Clinical and necropsy findings showed the presence of diarrhea. Although early washing significantly lessened the discharge noted after 24 hours in two rabbits, the investigators indicate *that 20 mL of water was not sufficient to ensure that all the powdery test material as completely washed out of the treated eye.* In short, the most significant finding was that of corneal opacity which did not always clear by day 14. In this study, "Gypsy Moth Virus" was judged to be a moderate eye irritant, and the test material was judged not to be corrosive.	Gordon and Kinsel 1977 MRID 00068404 Litton Bionetics 1977

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

Product	Species/Exposure	Observations	Reference
OCULAR *(continued)*			
"Insect Virus *L. dispar* NPV Bioserv Lot #33"	Administration of 3 mg of test material in left eye of each of six New Zealand albino rabbits (weighing 2.0-2.5 kg). Right eyes served as controls. Rabbits were separated into 3 groups with 2 animals/group: 1 minute wash; 5 minute wash; and no wash. Treated eyes were scored at 24, 48, and 72 hours and at 4 and 7 days after treatment.	Slight conjunctival irritation was observed at 24 hours in the two rabbits in the "no wash" group, but the irritation cleared at 48 hours. No irritation was observed when the test material was washed out of the eyes at 1 minute and 5 minutes. The irritation observed in the "no wash" group was not considered to be significant by the investigators.	Becker and Parke 1976c MRID 00068403 Cannon Labs 1976e
L. dispar NPV (Bioserv Lot #33)	Administration of 20 mL test compound to left eye of each of six New Zealand white rabbits (weight range of 2.0-2.5 kg). Right eyes served as controls. Treated eyes were observed and scored at 24, 48, and 72 hours and 4 and 7 days after exposure.	Positive reaction in all six rabbits at 24, 48, and 72 hours and 4 and 7 days. 4/6 animals had corneal involvement at 24, 48, and 72 hours and 4 and 7 days. Conjunctival involvement was present at 24, 48, and 72 hours and 4 and 7 days.	Becker and Parke 1976a MRID 00060696
Gypchek TGAI (Gypchek *Lymantria dispar* NPV) (Lot GR-14A) wettable powder	New Zealand white rabbits, 6 males and 6 females received undiluted test substance (0.1 mL by volume) in the conjunctival sac of the right eye. Three treated eyes were each washed with deionized water for 1 minute, beginning 30 seconds after treatment. Three treated eyes were left unwashed for 24 hours.	In the unwashed eyes, the maximum average irritation score was 37.3 and was reached at 24 hours after exposure. Gypchek TGAI in unwashed eyes was rated *moderately irritating*. Fluorescein staining, which was observed in all six treated unwashed eyes at 24 hours, was not observed in any eyes on day 17. In washed eyes, the maximum average irritation score was 5.3 and was reached at 1 hour after treatment. Gypchek TGAI in washed eyes was rated *minimally irritating*. Fluorescein staining was not observed in any of the treated washed eyes.	Kuhn 1997a MRID 44354301

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

Product	Species/Exposure	Observations	Reference
OCULAR *(continued)*			
Gypchek Solution 2X (Gypchek *Lymantria dispar* NPV) (Lot GR-14A) wettable powder	New Zealand white rabbits, 3 males and 3 females received a dose of 0.1 mL of the test substance mixed with sterile water in the conjunctival sac of the right eye. All treated eyes were washed with deionized water for 1 minute immediately after recording the 24-hour observation.	No positive effects were observed in any of the treated eyes at any time during the study. Gypchek Solution 2X was rated *non-irritating* with a maximum irritation score of 0.0. See Section 3.1.11 for additional discussion.	Kuhn 1997b MRID 44354302
LDP 53 air dried sample ($3.73x10^{10}$ PIBs/g)	Adult New Zealand albino rabbits (weighing between 2.0 and 2.5 kg) 3 rabbits/test group, received 50 mg of "LDP 53" in the right eye with the untreated eye serving as a control. The test groups were treated as follows: Group I: 10 second wash; Group II: 1 minute wash; Group III: 5 minute wash; and Group IV: no wash. The treated eyes were observed and scored at 24, 48, and 72 hours as well as 4, 7, and 14 days after exposure. In addition, the treated and control eyes were swabbed before exposure and again at 4, 7, and 14 days after exposure for microbiological evaluation after a 48-hour incubation period.	In Group I (10 second wash), one rabbit had eye irritation limited to conjunctival redness that lasted through day 4. In Group II (1 minute wash), all three rabbits exhibited conjunctival redness of grade 2 at 24 hours and grade 1 at 48 hours. All irritation in this group cleared after 4 days. In Group III (5 minute wash) all three rabbits had corneal opacity of grade 1 throughout the test. Iritis was present in two rabbits throughout the test and in one rabbit for 4 days. Conjunctival irritation was present in all rabbits throughout the test. In Group IV (no wash), all three rabbits had corneal opacity, but one of the cases cleared after 48 hours while the remaining two exhibited corneal opacity throughout the study. Iritis cleared after 72 hours in one rabbit, after 7 days in another rabbit, and continued in the third rabbit for the duration of the test. Conjunctival irritation persisted in all three rabbits through day 14. Microbial evaluation revealed *Staph epidermidis*, *Corynebacteria xerosis*, *Bacillus cereus*, and *Bacillius subtillis*, but the findings were not considered to be significant.	Imlay and Terrell 1978 MRID 00091124 Cannon Labs 1978

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

Product	Species/Exposure	Observations	Reference
		INHALATION	
P. dispar[1] nuclear PIB's, Hamden Standard	Sprague Dawley rats (9 males and 9 females) exposed for 60 minutes (heads only) to 0.028 to 0.81 mg LdNPV/L.	No mortality and no evidence of toxicity resulting from exposure.	Thronett 1975 MRID 00049266 Litton Bionetics 1975d
L. dispar NPV (Lot #33)	Rats (10 males, weighing 125-146 g) exposed to average analytical concentration of 6.12 ± 2.087 mg/L for 1 hour. Recovery period of 14 days.	No mortality and no treatment-related effects on lung or trachea tissue. Appendix to the study in the open literature (Cannon Labs 1976c) indicates that alveolar thickening and a single finding of low grade pneumonitis were considered coincidental and not statistically significant by a pathologist at Cannon Labs who reviewed lung and trachea sections from the exposed rats.	Brown 1976 MRID 00060695 Cannon Labs 1976c
P. dispar[1] NPV intact polyhedra	Single virus dose exposure to (***intranasal instillation***) 0.02 g/animal polyhedra to adult mice [10 males (5 untreated and 5 immunosuppressed) and 10 females (5 untreated and 5 immunosuppressed)]. Immunosuppressed mice were *selectively depleted of cell-mediated immune function by thymectomy and treatment with anit-lymphoctye serum* (Cytoxan administered ip at 300 mg/kg/mouse). Positive controls treated with *autoclaved* polyhedra; negative controls treated with saline. All animals observed for 21 days.	Negative results. Serological results indicated that the animals with intact immune systems were exposed to NPV antigen, since positive reactions were apparent with autoclaved and non-autoclaved NPV preparations. Control (saline) exposure did not produce antibody responses. Investigators indicated that serology (characterization of *P. dispar*[1] NPV) and histopathology are incomplete.	Shope et al. 1975 MRID 000606700

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

Product	Species/Exposure	Observations	Reference
INHALATION ***(continued)***			
L. dispar NPV (BioServ Lot#33; $6.6x10^{10}$ PIBs/g as dust)	Rats, 10 males (initial weights of 125-146 g) exposed to *L. dispar* NPV via inhalation for 1 hour at a concentration of 6.12 ± 2.087 mg/L (= $4.04x10^{8}$ ± $1.38x10^{8}$ PIBs/L) for 1 hour and sacrificed 1, 7, or 14 days after exposure	Average persistence in lung tissue of sacrificed animals: day 1 sacrifice: 95.96% (190/198) day 7 sacrifice: 68.0% (68/100) day 14 sacrifice: 18.09 % (36/199)	USDA/FS 19??g MRID 00060701 USDA/FS 19??d MRID 00066105 USDA/FS 1975? MRID 00090598 [most complete discussion of protocol and results]
INTRAPERITONEAL			
L-Dispar. Lot 33	10 Male ICR mice weighing 18-25 g given single i.p. injection of 0.5 mL/mouse. To achieve dose, 50 mg of test material was suspended in 10 mL of saline or 5 mg/mL. Thus, the dose was about 2.5 mg LdNPV per mouse or about 125 mg/kg bw using an average bw of 0.02 kg.	No mortality and no adverse effects observed at 1,3, or 6 hours after treatment or at daily observations thereafter for 7 days.	Terrell and Parke 1976c MRID 00066103 Terrell and Parke 1976d MRID 00066109

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

Product	Species/Exposure	Observations	Reference
	OTHER		
P. dispar[1] NPV intact polyhedra	Single virus dose exposure (***footpad inoculation***, not otherwise specified) to 0.02 g/animal polyhedra to adult mice [10 males (5 untreated and 5 immunosuppressed) and 10 females (5 untreated and 5 immunosuppressed)]. Immuno-suppressed mice were *selectively depleted of cell-mediated immune function by thymectomy and treatment with anit-lymphoctye serum* (Cytoxan administered ip at 300 mg/kg/mouse). Positive controls treated with *autoclaved* polyhedra; negative controls treated with saline. All animals observed for 21 days.	Mice developed bacterial abscess *localized* at the site of inoculation, but showed no other signs of toxicity. The study does not specify whether the incidence of bacterial infection was different between immunosuppressed and immunocompetent mice.	Shope et al. 1975 MRID 000606700

[1] *P. dispar* refers to *Porthetria dispar*, a former designation for the gypsy moth.

Appendix 2: Toxicity of Gypsy Moth LdNPV to Birds

Product	Species/Exposure	Observations	Reference
		ORAL	
Gypsy Moth Virus (Lot #33) (NOS)	Mallard ducks (between 10 and 15 days old) 10/dose group exposed to dietary concentrations of LdNPV ranging from 0.1x to 100x field usage (i.e., $1.04x10^6$, $5.2x10^6$, $1.04x10^7$, $1.04x10^8$, $1.04x10^9$ PIBs/g of feed). Controls were not exposed to virus in the diet.	No signs of abnormal behavior such as decreased locomotor activity, feather erection, or loss of righting reflex. No mortality except for one death at the 1x level that was not considered to be treatment related.	Roberts and Wineholt 1976 MRID 00068410
NPV of the gypsy moth	Gypsy moth avian predators (6 black-capped chickadees, *Parus atricapillus*, and 9 house sparrows, *Passer domesticus*) fed LdNPV-infected 4^{th} instar gypsy moth larvae on day 1 and on alternate days for 3 weeks. Each infected larva contained from $3.3x10^7$ to $2.1x10^8$ PIB. During the test period, each chickadee ate 70-80 infected larvae (from $2.3x10^9$ to $1.7x10^{10}$ PIB) and each treated sparrow ate 90-100 infected larvae (from $3.0x10^9$ to $2.1x10^{10}$ PIB).	No signs of disease were observed in the birds during the test period; body weight and results of histological examination of organs of treated birds indicated that LdNPV exposure caused no apparent short-term adverse effects.	Podgwaite and Galipeau 1978 MRID 00134318

Appendix 2: Toxicity of Gypsy Moth LdNPV to Birds

Product	Species/Exposure	Observations	Reference
FIELD STUDIES			
NPV molasses-based formulation containing "k" rotor purified polyhedral inclusion bodies (PIBs) (0.25 gal Cargill insecticide base; 6.0 oz Chevron spray sticker; 1.0 lb IMC 900001; 1.75 gal water)	Resident songbird populations, caged quail (*Colinus virginianus*) in woodland plots in central mountain region of Pennsylvania treated with two aerial applications (May 28 and June 2, 1975) of LdNPV at the rate of $2.5x10^{12}$ PIBs/ha (18.7 L/ha). Applications were made with 450 hp Grumman AgCat aircraft equipped with 6 Beecomist nozzles. Elevations of treated plots ranged from 1500 to 1800 ft (550-650 m) above sea level and supported 300-2000 egg masses/acre (750-5000/ha). Untreated plots were used as a negative control.	No significant differences in population trends between treated and control plots at either 1 or 2 months after LdNPV applications. LdNPV treatment had no adverse effects on the resident song birds or caged quail. *In fact, it appeared that the LdNPV application, by reducing defoliation, helped to maintain significantly higher densities of the yellow throat warblers; once bird species which utilizes a niche close to the ground.* Investigators concluded that aerial application of LdNPV at the rates used in this study had no adverse effects on birds that use gypsy moths as a food source or birds that contact the virus from the LdNPV spray, spray residue, or the dying larvae.	Lautenschlager et al. 1976b MRID 00066108 Lautenschlager et al. 1978b MRID 00134316 [This is an abstract of the Lautenschlager et al. 1976b study that was submitted separately to EPA] Lautenschlager and Podgwaite 1979b
NPV formulation containing a commercial adjuvant and "k" rotor purified PIBs (1.0 gal Sandoz Virus Adjuvant; 1.0 gal water).	Resident songbird populations caged quail (*Colinus virginianus*) in woodland plots in central mountain region of Pennsylvania treated with two aerial applications (May 28 and June 2, 1975) of LdNPV at the rate of $2.5x10^{12}$ PIBs/ha (18.7 L/ha). Applications were made with 450 hp Grumman AgCat aircraft equipped with 6 Beecomist nozzles. Elevations of treated plots ranged from 1500 to 1800 ft (550-650 m) above sea level and supported 300-2000 egg masses/acre (750-5000/ha Untreated plots were used as a negative control.	No significant differences in population trends between treated and control plots at either 1 or 2 months after LdNPV applications. LdNPV treatment had no adverse effects on the resident song birds or caged quail. *In fact, it appeared that the NPV application, by reducing defoliation, helped to maintain significantly higher densities of the yellow throat warblers; once bird species which utilizes a niche close to the ground.* Investigators conclude that aerial application of LdNPV at the rates used in this study had no adverse effects on birds that use gypsy moths as a food source or birds that contact the virus from the LdNPV spray, spray residue, or the dying larvae.	Lautenschlager et al. 1976b MRID 00066108 [This is the same study as above but using a different formulation of LdNPV] Lautenschlager et al. 1978b MRID 00134316 Lautenschlager and Podgwaite 1979b

Appendix 3: Gypsy Moth NPV Toxicity in Nontarget Terrestrial Insects

Product	Species/Exposure	Observations	Reference
*Ld*NPV (aqueous suspension)	46 species of nontarget Lepidoptera exposed to four successive 24- to 48-hour doses of $3x10^4$ PIBs in 2µL applied to pellets of artificial diet or isolated surfaces of foliage	No statistically significant mortality, compared with controls; 0.0% infection in all treated species.	Barber et al. 1993
*Ld*NPV (aqueous suspension)	Adult fly, *Cyrtophleba coquilletti* Aldr. exposed to single dose of $12x10^5$ PIBs in 2µL of 30% sucrose solution. Those that completely consumed the dose were transferred to appropriate maintenance conditions for 7-10 days and then frozen.	No statistically significant motality, compared with controls; 0.0% infection.	Barber et al. 1993
*Ld*NPV (aqueous suspension)	Adult male bees, *Megachile rotundata* (Fabr). exposed to single dose of $12x10^5$ PIBs in 2µL of 30% sucrose solution. Those that completely consumed the dose were transferred to appropriate maintenance conditions for 7-10 days and then frozen.	No statistically significant motality, compared with controls; 0.0% infection.	Barber et al. 1993
Gypsy Moth NPV *Porthetria dispar* (L).	Adult honey bees exposed to estimated dose of $1x10^6$ polyhedra in sucrose solution	No indication of detrimental effects resulting from exposure to test substance.	Cantwell et al. 1972
Gypsy Moth NPV (*Porthetria dispar*)	Honeybee (*Apis melliferai*) in observation hives fed $10x10^9$ polyhedra mixed with 200 mL sucrose solution (sugar-water 1:1) (total dose/hive) over 4-month period.	No differences were observed between treated and untreated bee colonies	Knox 1970
Gypchek	Application at a rate of $8x10^{10}$ PIB/ha on ant communities. Pitfall traps operated for 45 weeks during summers of 1995-1997 in George Washington national Forest, Augusta County, VA and Monongahela National Forest in Pocahontas County, WV.	Ants representing 17 genera and 31 species were collected, indicating that species richness, diversity, abundance, and species composition were not adversely affected by treatment.	Wang et al. 2000

Appendix 4: Toxicity of NPV to Aquatic Invertebrates			
Product	Species/Exposure	Observations	Reference
NPV containing $1.7x10^{11}$ polyhedra/g and some bacterial impurities.	Daphnia (*D. magna*), 15, ≤24 hours old exposed to test concentration of 250 polyhedra/g. Virus was added initially and anew every 2 days. Complete experiment was replicated 3x (conducted several weeks apart in time). Surviving, mature *Daphnia* produced young, which were counted.	Treatment had no significant effect on either survival ($p>0.05$) or reproduction ($p>0.05$).	Streams 1976 MRID 00068408
NPV containing $1.7x10^{11}$ polyhedra/g and some bacterial impurities.	Daphnia (*D. magna*) surviving the acute toxicity study were randomly frozen for bioassay or transferred to a virus-free medium with samples taken at 6- to 12-hour intervals. *The purpose of the bioassays was to determine whether NPV could be detected in a apparently healthy Daphnia reared in water with a high concentration of polyhedra and , if so, how soon the NPV disappeared from Daphnia when placed in a virus free medium.*	The average mortality rate for gypsy moth larvae fed *Daphnia* reared in virus-treated water was similar to that of larvae fed *Daphnia* reared in virus free water (2.2% vs.3.1%); the average percent mortality rate for gypsy moth larvae fed a sterile diet was 0.5%. Mortality rate was not affected when gypsy moth larvae were fed *Daphnia* removed from virus-treated medium and reared in virus free medium for up to 48 hours. *Daphnia* did not accumulate gypsy moth NPV under the test conditions.	Streams 1976 MRID 00068408
NPV containing $1.7x10^{11}$ polyhedra/g and some bacterial impurities.	Backswimmers (*Notonecta undulata*), newly hatched nymphs reared for the first 2 instars in virus-free water after which time NPV at a concentration of 250 polyhedra/mL was added to the containers. The treated backswimmers were fed live, virus-treated *Daphnia*. The *Daphnia* fed to the treated backswimmers were reared in water containing virus at a concentration of 250 polyhedra/mL and the treated water was renewed about 3x/week.	No significant effects of NPV on *N. undulata* were observed with regard to survival or reproduction. Data are presented in Table 3 of the study. Bioassay results are recorded in Table 7 of the study and indicate that *N. undulata* reared in water with 250 polyhedra/mL of gypsy moth NPV or fed *Daphnia* reared in similar concentrations do not accumulate the NPV virus.	Streams 1976 MRID 00068408

Appendix 4: Toxicity of NPV to Aquatic Invertebrates			
Product	Species/Exposure	Observations	Reference
NPV containing $1.7x10^{11}$ polyhedra/g and some bacterial impurities.	Midge (*Chironomus thummi*), newly hatched larvae reared to pupation in containers in which NPV was mixed with the water and the food at a concentration of 250 polyhedra/mL. Emerging adults were set up in screened breeding cages for 1 week to obtain reproduction and to check on the viability of any eggs produced.	No significant difference ($p>0.05$) in survival of treated midge, compared with controls; developmental time was identical in treated and in untreated replicates; and reproduction by adults reared from treated replicates was similar to that observed in controls (all egg masses were fertile).	Streams 1976 MRID 00068408
NPV containing $1.7x10^{11}$ polyhedra/g and some bacterial impurities.	Water boatmen (adult *Hesperocorixa interrupta* [n=10/replicate] and *Sigara gordita* n=20/replicate]) exposed to NPV at a concentration in water of 250 polyhedra/mL for 4 weeks.	No significant difference in survival of either species in among treated and control adults and no apparent adverse effects on reproduction were observed in *Sigara*, which produced eggs, many of which hatched before the end of the study. Results of the bioassay indicate that the water boatmen did not accumulate NPV under the conditions of the study.	Streams 1976 MRID 00068408

Appendix H
Disparlure
Risk Assessment

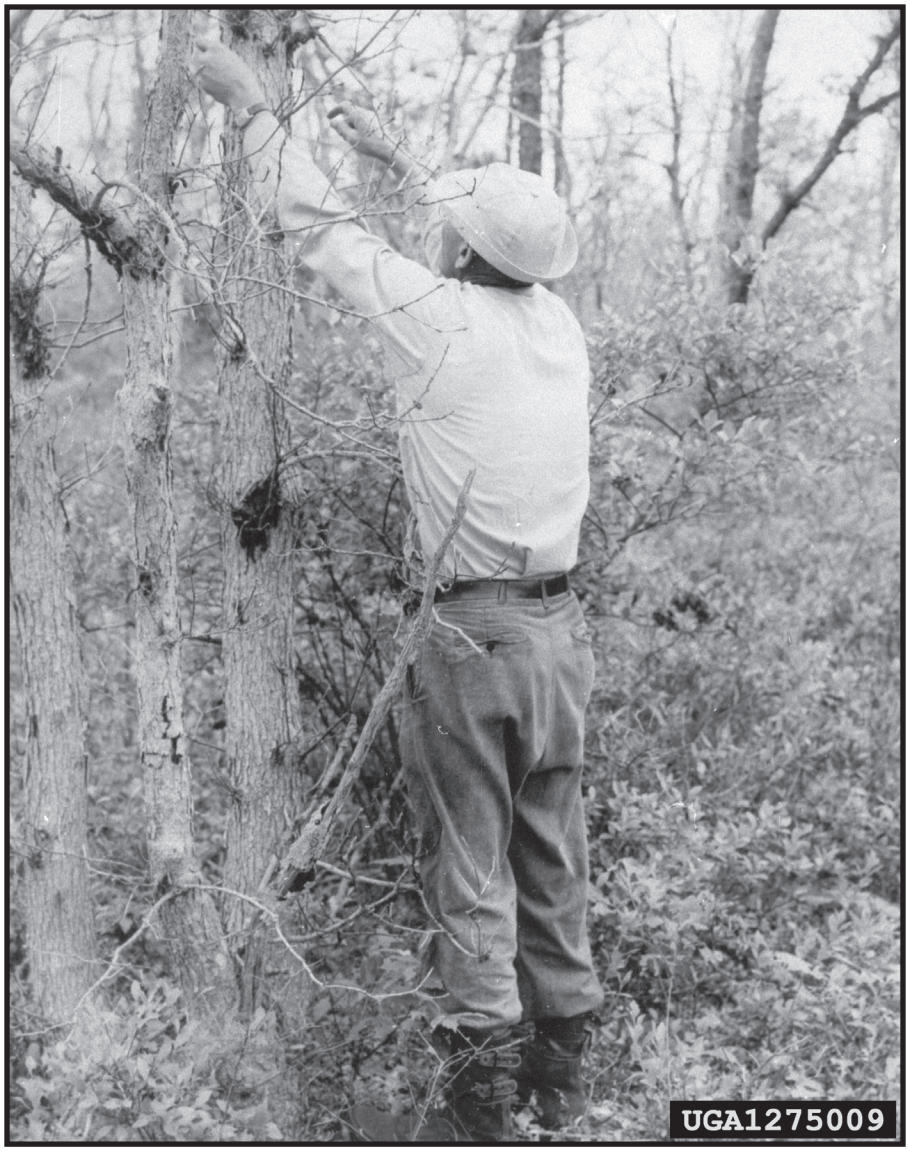

Figure H-1. Female gypsy moth pupae were gathered in Massachusetts in 1948 in order to obtain sex attractant for trapping programs.

SERA TR 06-52-07-02d

Control/Eradication Agents for the Gypsy Moth - Human Health and Ecological Risk Assessment for Disparlure (a.i.) and Disrupt II formulation – FINAL REPORT

Prepared for:

USDA, Forest Service

Forest Health Protection

USDA Forest Service Contract No: **AG-3187-C-06-0010**
USDA Order No. **AG-43ZP-D-06-0021**
SERA Task No. **52-07**

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August 28, 2006 (Risk Assessment)
July 20, September 10, and September 19, 2007 (Editorial Corrections)

PREFACE

This document is a revision to a risk assessment that was originally prepared by Syracuse Environmental Research Associates, Inc. (SERA Inc.) under GSA Contract No. GS-10F-0082F, USDA Forest Service BPA: WO-01-3187-0150, USDA Purchase Order No.: 43-3187-1-0269. The SERA documented was prepared by Drs. Patrick R. Durkin (SERA Inc.) and Julie Klotzbach (currently with Syracuse Research Corporation). The SERA document was submitted to the USDA Forest Service as Control/Eradication Agents for the Gypsy Moth - Human Health and Ecological Risk Assessment for Disparlure (a.i.) - FINAL REPORT, SERA TR 04-43-05-04b, reported dated August 27, 2004. As indicated in the title, SERA TR 04-43-05-04b covered only the active ingredient – i.e., disparlure – and did not address the formulation of disparlure in Disrupt II flakes. The original SERA document was reviewed by Dr. Rolf Hartung (Univ. Michigan, retired) and by USDA/Forest Service personnel: Dr. Paul Mistretta, Mr. Joseph Cook, and Ms. Donna Leonard.

Under USDA Order No. AG-43ZP-D-06-0015, USDA Forest Service Contract No: AG-3187-C-06-0010, SERA revised the above report to include Disrupt II flakes. The subsequent revision (SERA TR 06-52-02-01a) was submitted to the USDA on June 30, 2006). This revision was based on new information provided by the USDA/Forest Service. The listing below indicates the specific references that were added to the June 30, 2006 revised risk assessment concerning Disrupt II:

Hercon Environmental. 2006a. Hercon Disrupt II Product Label. Copy courtesy of Donna Leonard, USDA Forest Service, Forest Health Protection, PO Box 2680, Asheville, NC 28802. e-mail: dleonard@fs.fed.us. Received June 27, 2006.

Hercon Environmental. 2006b. Hercon Disrupt II Material Safety Data Sheet. Copy courtesy of Priscilla MacLean, Product Development Manager, Hercon Environmental, P.O. Box 435, Emigsville PA, 17318. e-mail: pmaclean@herconenviron.com. Received June 27, 2006.

Leonard D. 2006a. Comments on Application Rates for Disparlure in STS (Slow-The-Spread) Programs. Comments by Donna Leonard, USDA Forest Service, Forest Health Protection, Asheville, NC. Comments received via email from dleonard@fs.fed.us on June 27, 2006.

Leonard D. 2006b. Comments on The Use of Disparlure in STS (Slow-The-Spread) Programs. Comments by Donna Leonard, USDA Forest Service, Forest Health Protection, Asheville, NC. Comments received via email from dleonard@fs.fed.us on June 27, 2006.

MacLean P. 2006. Comments on Inerts in Disrupt II, Product Development Manager, Hercon Environmental, P.O. Box 435, Emigsville PA, 17318. e-mail: pmaclean@herconenviron.com. Received June 27, 2006.

Palmer SJ; Krueger HO. 2006a. SF 2003 and SF 2005: A 48-Hour Static-Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*). Wildlife International, Ltd. Project Number: 6 L4a- 102. Study completion date: Jan. 12, 2006. Copy courtesy of Paul Mistretta, USDA/FS.

Palmer SJ; Krueger HO. 2006b. MF 2003 and MF 2005: A 48-Hour Static-Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*). Wildlife International, Ltd. Project Number: 6 L4a- 101. Study completion date: Jan. 12, 2006. Copy courtesy of Paul Mistretta, USDA/FS.

Because of limitations in the available toxicity data on disparlure and Disrupt II, more extensive use has been made of quantitative structure activity relationships (QSAR) and the following additional references (not specific to disparlure) have been added:

Bintein S, Devillers J, and Karcher W. 1993. Nonlinear dependence of fish bioconcentration on n-octanol/water partition coefficient. SAR QSAR Environ Res. 1(1):29-39.

Clements RG, Nabholz JV, and Zeeman M. 1996. Estimating Toxicity of Industrial Chemicals to Aquatic Organisms Using Structure-activity Relationships. Environmental Effects Branch, Health and Environmental Review Division, Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency. Report dated August 30, 1996.

Jeppsson R. 1975. Parabolic Relationship between Lipophilicity and Biological Activity of Aliphatic Hydrocarbons, Ethers and Ketones after Intravenous Injections of Emulsion Formulations into Mice. Acta Pharmacol. Et Toxicol. 37: 56-64.

U.S. EPA/OPPT (U.S. Environmental Protection Agency/Office of Pollution Prevention and Toxics). 2000. On-Line EPI Suite User's Guide, Version 3.12. Developed by the EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC). Available at: http://www.epa.gov/opptintr/exposure/docs/episuite.htm

SERA TR 06-52-07-01a was then submitted based on comments from Forest Service and APHIS personnel. A consolidation of comments was prepared by Joe Cook (USDA/FS). This was the primary source for the current revisions. Comments from various Forest Service personnel were provided and consulted as needed, including comments from Hank Appleton, Jesus Cota, John Kyhl, and Donna Leonard. A PDF copy of the risk assessment with annotations from APHIS personnel was also consulted. Lastly, an unpublished synopsis of the following study was provided by Donna Leonard, reviewed and incorporated into this risk assessment as appropriate:

Thwaits BF; Sorensen PW. 2005. Olfactory sensitivity of rainbow trout to racemic disparlure. Unpublished synopsis dated April 1, 2005. Copy courtesy of Donna Leonard, USDA/Forest Service. 2 pp.

The current report, SERA TR 06-52-07-02a, is based on editorial comments from Joe Cook, some additional comments on formulations from Donna Leonard (cited as Leonard 2006e), and internal review. There are no substantial technical changes from SERA TR 06-52-07-01a.

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LIST OF APPENDICES

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Workbook
Disparlure: Simplified EXCEL Worksheets for Calculating Risks to Small Aquatic Invertebrates SERA EXWS 06-52-07-01a. Worksheet dated August 25, 2006.

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AEL	adverse-effect level
AGM	Asian Gypsy Moth
a.i.	active ingredient
BCF	bioconcentration factor
bw	body weight
CBI	confidential business information
cm	centimeter
CNS	central nervous system
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
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FQPA	Food Quality Protection Act
g	gram
ha	hectare
HQ	hazard quotient
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
m	meter
M	male

ACRONYMS, ABBREVIATIONS, AND SYMBOLS (*continued*)

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
NAGM	North American Gypsy Moth
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
OPP	Office of Pesticide Programs
OPPTS	Office of Pesticide Planning and Toxic Substances
ppm	parts per million (used in expressing dietary concentrations only)
QSAR	quantitative structure activity relationship
RfD	reference dose
SERA	Syracuse Environmental Research Associates
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
WHO	World Health Organization
µ	micron
▸	greater than
≥	greater than or equal to
<	less than
≤	less than or equal to
=	equal to
≃	approximately equal to
~	approximately

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)	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
1×10^{-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

Disparlure is a naturally occurring insect pheromone used to disrupt mating of gypsy moths by confusing male moths. Disparlure is also used as an attractant in traps. There are limited data available on the toxicity of disparlure. Only a small number of acute exposure studies have been conducted; no chronic toxicity studies in any species were identified in the available literature. Based on the results of the available data, the toxicity profile of disparlure in terrestrial animals does not suggest that disparlure is likely to cause adverse effects at plausible levels of exposure. Similarly, disparlure is not likely to cause any toxic effects in aquatic species at the limit of solubility of disparlure in water. Thus, under normal conditions of exposure, no hazard to aquatic species can be identified. In cases of an accidental application of disparlure to a small body of standing water, such as a pond, no effects are likely in fish. An accidental application or some other similar event such as an accidental spill could lead to an insoluble film of disparlure at the air-water interface of a standing body of water. This could result in some small invertebrates becoming trapped in the film of disparlure. While the entrapment of daphnids has been observed in laboratory studies of both disparlure and Disrupt II formulations, the likelihood of this occurring in the field to an extent that detectable effects would be observed is difficult to determine. The formation of a film that could trap small invertebrates in rapidly moving bodies of water does not seem plausible.

PROGRAM DESCRIPTION

Disparlure is a naturally occurring insect pheromone (attractant) synthesized by the female gypsy moth to attract the male gypsy moth. Disparlure can take two enantiomer forms, referred to as (+)disparlure and (-)disparlure. Enantiomers are mirror-image molecules with identical gross structures. The (+)enantiomer is the form produced by the female gypsy moth and is the only form that is biologically active as an attractant. In gypsy moth programs, two forms of disparlure are used: the (+)enantiomer and the racemic mixture, a 50:50 blend of the (+)enantiomer and (-)enantiomer. Racemic disparlure is used as a control agent. It is broadcast over relatively large areas and disrupts mating by confusing male moths – i.e., the male moth has difficulty in locating the female moth.

Disparlure is always formulated in a slow release matrix and several different formulations have been tested including polyvinyl chloride flakes, microcapsules, and polyvinyl chloride twine. Disrupt II, a formulation of disparlure in polyvinylchloride flakes, has been used by the USDA Forest Service for many years. The specific formulation has evolved over time. This risk assessment considers the available information both on the current and some previous Disrupt II formulations. As noted by Leonard (2006e), it is possible that the U.S. EPA will require different labels for the two different Disrupt formulations, with the previous formulation designated as Disrupt II and the newer formulation designated as Disrupt III. Because this decision has not yet been made, this risk assessment will refer to the older Disrupt formulation as *standard flakes* and the newer Disrupt formulation as *modified flakes*. These designations are discussed further in Section 4.1.3.3 in terms of differences in toxicity to *Daphnia*.

Since 1995, the use of disparlure in programs intended to slow the spread of gypsy moths has increased over 250-fold, from 2,448 acres treated in 1995 to a maximum of 647,394 acres treated in 2003. The (+)enantiomer of disparlure is used as an attractant or bait in two types of traps: milk carton traps that also contain DDVP and delta traps that do not contain an insecticide. These traps are used to monitor existing (endemic) populations and detect new infestations.

HUMAN HEALTH RISK ASSESSMENT

Hazard Identification – Insect pheromones are generally regarded as nontoxic to mammals and these pheromones are commonly employed in very low environmental concentrations. Consequently, U.S. EPA requires less rigorous testing of these products than is required of insecticides. Except for some standard acute toxicity studies in laboratory mammals, few data are available regarding the toxicity of disparlure to terrestrial species. Results of acute exposure studies for oral, dermal, ocular and inhalation exposure to disparlure show no indication of adverse effects. The LD_{50} of a single dose administered to rats by gavage exceeds 34,600 mg/kg. With the exception of one acute gavage study in rats using the 50:50 racemic mix, none of the toxicity studies specified whether the 50:50 racemic mix or the (+)enantiomer was tested. Based on the results of studies on disparlure itself (i.e., the active ingredient), acute exposure to disparlure has very low toxicity in mammals. 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. There is no information available regarding the kinetics and metabolism of disparlure in mammals. The kinetics of absorption of disparlure following dermal, oral or inhalation exposure are not documented in the available literature. A case report of an accidental exposure indicates that disparlure may persist in humans for years.

Exposure Assessment – For both occupational exposure of workers and accidental exposure of the general public, exposure to disparlure may involve multiple routes of exposure (i.e., oral, dermal, and inhalation). Nonetheless, dermal exposure is generally most likely to be the predominant route. While exposure scenarios can be developed and exposures quantified for each potential exposure route based on application rates of disparlure and limited monitoring data, given the low toxicity of disparlure to laboratory mammals and the lack of chronic toxicity studies, detailed quantitative estimates of exposure will not significantly add to the assessment of risk associated with disparlure.

Dose-Response Assessment – The toxicity data on disparlure are not adequate for making a standard dose-response assessment. The limited available data indicate that disparlure has a low order of acute toxicity based on mortality as follows: oral LD_{50} >34,600 mg/kg, dermal LD_{50} >2,025 mg/kg, and inhalation LC_{50} >5 mg/L x 1 hour. Data regarding the toxicity of disparlure to animals or humans after subchronic or chronic exposures were not located. Moreover, the acute toxicity of this compound for endpoints other than mortality is poorly characterized. Thus, due to insufficient data, the U.S. EPA has not derived either an RfD for acute or chronic exposure.

Risk Characterization – Although studies on the acute toxicity of disparlure have been conducted in laboratory animals, the lack of subchronic or 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 that 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 to 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 low application rates and the nature of plausible exposures of humans to disparlure.

ECOLOGICAL RISK ASSESSMENT

Hazard Identification – There is very little information regarding the toxicity of disparlure to nontarget wildlife species. As discussed above, rigorous toxicity testing of disparlure has not been required by the U.S. EPA. Thus, the only studies available are acute toxicity studies in bobwhite quail, mallard ducks, rainbow trout, bluegill sunfish, *Daphnia magna* and Eastern oysters. No chronic toxicity studies were identified in the literature or in the studies submitted to the U.S. EPA.

Results of acute gavage and dietary toxicity 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 bw in bobwhite quail.

Limited data are available regarding the toxicity of disparlure to aquatic animals. A major issue in the interpretation of the aquatic toxicity data on disparlure involves the solubility of disparlure in water. While no measured values for the solubility of disparlure in water are available, estimates based on quantitative structure-activity relationships developed by the U.S. EPA suggest that the solubility of disparlure in water is in the range of 0.0019 to 0.0028 mg/L. The bioassays that have been conducted on disparlure and Disrupt II formulations of disparlure have not measured concentrations of disparlure in the test water but report nominal concentrations of disparlure that exceed the water solubility of disparlure by factors of about 10 [0.028 mg/L] to over 150,000 [300 mg/L]. Based on the results of the available bioassays and considerations of water solubility, disparlure does not appear to present any toxic hazards to aquatic species. In toxicity tests of small aquatic invertebrates (i.e., daphnids), trapping of the organism at the surface of the water has been noted in bioassays of both technical grade disparlure and Disrupt II formulations. The trapping of small invertebrates at surface of the water can present a physical hazard to the organism. The significance of this physical hazard observed in bioassays to potential hazards in field applications is unclear.

Exposure Assessment – Disparlure appears to be essentially nontoxic to mammals and birds. While this assessment is limited by the lack of chronic toxicity data in terrestrial species, it is not expected that acute or chronic exposure of terrestrial mammals or birds to disparlure would result

in the development of significant adverse effects. Given the low toxicity of disparlure and limited available data, an exposure assessment for terrestrial species would not add to the assessment of risk for terrestrial species. Thus, an exposure assessment for terrestrial species is not included in this risk assessment. For aquatic species, the range of plausible nominal concentrations of disparlure in water are calculated at 0.0015 mg/L to 0.0037 mg/L over the range of application rates considered in this risk assessment. These concentrations apply to a 1 meter deep body of water. The lower end of this range is within the estimated solubility of disparlure in water – i.e., 0.0019 to 0.0028 mg/L.

Dose-Response Assessment – Given the low toxicity of disparlure to terrestrial animals coupled with the limitations imposed due to lack of chronic toxicity data, no standard dose-response can be made for disparlure for terrestrial species. Disparlure is produced by other species in the genus *Lymantria* that are closely related to the gypsy moth (http://www.pherobase.com) such as the nun moth (*Lymantria monacha*), a Eurasian pest of conifers that is considered a serious risk for introduction into North America (http://www.na.fs.fed.us/spfo/pubs/pest_al/nunmoth/nun_moth.shtm). However, since 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 cannot be made. Similarly, no explicit dose-response relationship is proposed for fish. There is no basis for asserting that adverse effects in fish are plausible under any foreseeable conditions. For aquatic invertebrates, there is no basis for asserting that toxic effects are likely at the limit of the solubility of disparlure in water. At nominal concentrations that exceed the solubility of disparlure in water (e.g., as the result of an accidental spill or application to water), small invertebrates that may interact with the water-surface interface could become trapped in this interface due to a layer of undissolved disparlure at the air-water interface.

Risk Characterization – There is little data available on terrestrial and aquatic animals to allow for a quantitative characterization of risk. Furthermore, the lack of chronic toxicity data in any species adds significant 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 assessed. Nonetheless, given the low toxicity of disparlure based on acute toxicity studies, it is unlikely that exposure to disparlure will result in the development of serious adverse effects in terrestrial and aquatic species. Regarding potential effects on terrestrial invertebrates, disparlure is able to disrupt mating of some other closely related species of moths other than the gypsy moth. These other closely related species, however, are all Asian or Eurasian species and are not known to exist in North America. Thus, there is no basis for asserting that mating disruption is plausible in nontarget species in North America.

Under normal conditions, aquatic species will not be exposed to substantial levels of disparlure. At the limit of the solubility of disparlure in water, there is no indication that toxic effects are likely in any aquatic species. If Disrupt II flakes are accidently applied to water, the amount of disparlure in the water could result in the formation of an insoluble layer of disparlure at the air-water interface. There is no indication that this would impact fish. Based on toxicity studies conducted in the laboratory, small invertebrates that come into contact with the air-water interface might become trapped in an insoluble film of disparlure. The likelihood of this occurring and the likelihood of this causing any detectable impact in a body of water is difficult

to determine and would vary with the quantity of flakes applied to the body of water and the depth of the body of water. Based on variability in the experimental data as well as the range of application rates used in the USDA programs, hazard quotients would vary from about 0.15 to about 0.37 below the level of concern by factors of about 3 to 10. This risk characterization applies to accidental application of disparlure to a 1 meter deep body of water.

1. INTRODUCTION

The USDA Forest Service uses disparlure and the formulation of disparlure as Disrupt II in programs to control or eradicate gypsy moth populations. This document is an update to a risk assessment prepared in 1995 (USDA 1995) 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 disparlure, 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 2006).

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. No published reviews regarding human health or ecological effects of disparlure have been encountered. Moreover, almost all of the mammalian toxicology studies and most of the ecotoxicology studies are unpublished reports submitted to the U.S. EPA as part of the registration process for disparlure.

Because of the lack of a detailed, recent review concerning disparlure and the preponderance of unpublished relevant data in U.S. EPA files, a complete search of the U.S. EPA FIFRA/CBI files was conducted. Full text copies of relevant studies were kindly provided by the U.S. EPA Office of Pesticide Programs. These studies were reviewed, discussed in Sections 3 and 4 as necessary, and synopses of the most relevant studies are provided in the appendices to this document.

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

2. PROGRAM DESCRIPTION

2.1. OVERVIEW

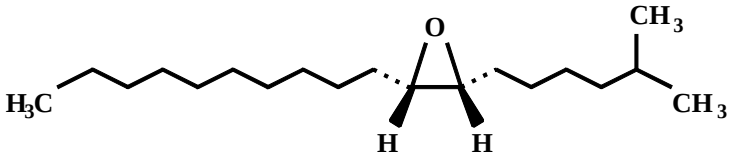
Disparlure is a naturally occurring insect pheromone (attractant) synthesized by the female gypsy moth to attract the male gypsy moth. Disparlure can take two enantiomer forms, referred to as (+)disparlure and (-)disparlure. Enantiomers are mirror-image molecules with identical gross structures. The (+)enantiomer is the form produced by the female gypsy moth and is the only form that is biologically active as an attractant. In gypsy moth programs two forms of disparlure are used: the (+) enantiomer that is used as an attractant or bait in traps and the racemic mixture, a 50:50 blend of the (+) and (-) enantiomers that is used as a control agent. When it is used as a control agent, racemic disparlure is broadcast over relatively large areas to disrupt mating by confusing the male moths.

Disparlure is always formulated in a slow release matrix and several different formulations have been tested including polyvinyl chloride flakes, microcapsules, and polyvinyl chloride twine. Disrupt II, a formulation of disparlure in polyvinylchloride flakes, has been used by the USDA Forest Service for many years. The specific formulation has evolved over time. This risk assessment considers the available information both on the current and some previous Disrupt II formulations.

Since 1995, the use of disparlure in programs intended to slow the spread of gypsy moths has increased over 250-fold, from 2,448 acres treated in 1995 to 647,394 acres treated in 2003. (+)disparlure is used as an attractant or bait in two types of traps: milk carton traps that also contain DDVP and delta traps that do not contain an insecticide. These traps are used to monitor existing (endemic) populations and detect new infestations.

2.2. CHEMICAL DESCRIPTION

Disparlure is the common name for cis-7,8-epoxy-2-methyloctadecane:



Disparlure can take two enantiomer forms, referred to as (+)disparlure and (-)disparlure. The term *enantiomer* refers to molecules that are structurally identical except for differences in the 3-dimensional configuration such that one form is the mirror image of the other.

(+)Disparlure is a naturally occurring insect pheromone (attractant) synthesized by the female gypsy moth to attract the male gypsy moth. (+)Disparlure is also a natural constituent of and is a pheromone for other species including the nun moth (*Lymantria monacha*, Morewood et al. 1999, 2000) and *Lymantria fumida* [the pink gypsy moth which is a species native to Japan]

(Schaefer et al. 1999). 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.

Selected chemical and physical properties of disparlure are summarized in Table 2-1. Due to the lack of experimental data, most of the values given in Table 2-1 are estimated from EPI Suite, an estimation program developed by Meylan and Howard (2000) in conjunction with the U.S. EPA (U.S. EPA/OPPT 2000). For convenience, the specific estimates for disparlure that were obtained from EPI Suite are referenced in this document as EPI Suite (2006) and a full copy of this run is included as Appendix 4.

In gypsy moth programs, two forms of disparlure are used: the (+)enantiomer and the racemic mixture, a 50:50 blend of the (+)enantiomer and (-)enantiomer. For disparlure, the (+)enantiomer is the biologically active form (that is, the form that attracts the male gypsy moth). Racemic disparlure is used as a control agent. It is broadcast over relatively large areas and disrupts mating by confusing male moths. This product is typically aerially applied in a single application just before the emergence of adult gypsy moths. Although the label for Disrupt II allows a second application later in the season, operational programs never use a second application.

As discussed in Section 3 and Section 4, most toxicity studies conducted on disparlure do not specify whether the racemic mix or the (+)enantiomer of disparlure was tested. Except for the attractant effects of (+)disparlure, there is no clear indication that toxicity profiles differ between the (+)enantiomer of disparlure and the 50:50 racemic mix. For the purposes of this risk assessment, no distinction is made between (+)disparlure and the racemic mix. All references to the active ingredient (a.i.) refer to disparlure and do not distinguish between (+)disparlure and the 50:50 racemic mix.

When used as a control agent, disparlure is formulated in a slow release matrix and several different formulations have been tested including polyvinyl chloride flakes, microcapsules, and twine (Caro et al. 1977, 1981; Taylor 1982). In recent programs, the USDA used Disrupt II (Leonhardt et al. 1996) and this formulation is currently registered by U.S. EPA (Hercon Environmental 1993). This formulation contains 17.9% disparlure and 82.1% carrier flakes. Disrupt II flakes are about 1/32 inch by 3/32 inch and consist of polyvinyl chloride films, polyvinyl chloride resin and a plasticizer (Hercon Environmental 2004). The USDA has participated in the development of new formulations of disparlure in either new flake formulations developed by Hercon or new microcapsule formulation being developed by 3M (Leonard 2004).

Currently, the USDA has elected to use a new Disrupt II flake formulation (Leonard 2006a,b). As with past formulations of Disrupt II, this flake formulation contains 17.9% disparlure and 82.1% polyvinylchloride carrier flakes and other inerts (Hercon 2006a,b). As detailed further in Section 4.1.3.3, toxicity data are available on the current formulation of Disrupt II as well as a previous formulation. Available information on the inerts in Disrupt II is discussed in Section 3.1.14.

2.3. APPLICATION METHODS AND RATES

The application rates recommended on the label of Disrupt II (Hercon 2006a), range from 6 grams a.i./acre to 30 grams a.i./acre, corresponding to about 0.0132 lb a.i./acre to 0.066 lb a.i./acre[1 gram = 0.0022 lb (avdp)].

The USDA uses disparlure in two different types of programs: slow the spread and eradication. Slow the spread programs involve the control of the North American Gypsy Moth (NAGM), a species that is already established in the US. Slow the spread programs are typically administered by the USDA/Forest Service using application rates of 6 grams a.i./acre and occasionally using an application rate of 15 g a.i./acre. Tobin and Leonard (2006) have estimated that this range of application rates will result in the release of disparlure that is substantially greater than the amounts released by female gypsy moths during a major outbreak.

Eradication efforts are administered by USDA/APHIS (Animal and Plant Health Inspection Service). Eradication efforts are focused on the Asian strain of the gypsy moth (AGM) that is not known to be established in the United States as well as small and isolated infestations of the NAGM that could be eradicated. For purposes of exclusion and eradication, APHIS considers AGM to be a separate species from NAGM. With NAGM, eradication uses applications of up to 15 g a.i./acre. The maximum labeled application rate of 30 g a.i./acre has only been used once for AGM eradication. This application involved only 600 acres out of a total of approximately 2.5 million acres treated between 1995 and 2005 – i.e., less than 0.03% of the total acres treated.

Because the application rate of 30 g a.i./acre is used only rarely, the current risk assessment will explicitly consider application rates in the range of 6 grams a.i./acre and 15 g a.i./acre. If other application rates need to be considered in certain applications, the Worksheet A02 of the EXCEL workbook that accompany this risk assessment may be modified. This workbook is described in Section 4.4.2 of this risk assessment.

(+)Disparlure is used as an attractant or bait in two types of traps: milk carton traps that also contain DDVP and delta traps that do not contain an insecticide. These traps are used to monitor existing (endemic) populations and detect new infestations. Since the early 1980s, (+)disparlure has been formulated as 3 x 25 mm plastic laminates (two outer layers of 50 μm PVC with an inner polymeric layer containing 500 μg (+)disparlure).

2.4. USE STATISTICS

Use statistics for the number of acres treated with disparlure according to type of use are summarized in Table 2-2 (USDA/FS 2005). From 1995 to 2003, the use of disparlure to slow the spread of gypsy moths increased substantially. In 1995, 2,448 acres were treated with disparlure flakes and in 2003, 647,394 acres were treated; this is an increase in acres treated of over 250-fold. It is anticipated that slow the spread applications will typically entail about 500,000 acres per year and that these applications will account for 99.9% of all mating disruption applications (Leonard 2005a).

3. HUMAN HEALTH RISK ASSESSMENT

3.1 HAZARD IDENTIFICATION

3.1.1 Overview.

Insect pheromones are generally regarded as nontoxic to mammals (Jacobson 1976) and, as with disparlure, application rates of insect pheromone are generally very low – i.e., pheromones are active at very low concentrations. Consequently, U.S. EPA requires less rigorous testing of these products than is required of insecticides (U.S. EPA 1994). Except for some standard acute toxicity studies in laboratory mammals, little information is available regarding the biological activity of disparlure. The USDA has funded acute toxicity studies on disparlure during its development for use in the gypsy moth control program. The studies were conducted by Industrial Bio-test and were submitted to the U.S. EPA by Hercon Environmental Company as part of the registration package (Kretchmar 1972). Summaries of these studies are published in the open literature (Beroza et al. 1975).

Results of acute toxicity studies for oral, dermal, ocular and inhalation exposure to disparlure are summarized in Table 3-1. With the exception of one acute gavage study in rats using the 50:50 racemic mix (Coleman 2000), none of the toxicity studies specified whether the 50:50 racemic mix or the (+)enantiomer was tested. Based on the results of studies on disparlure, acute exposure to disparlure appears to pose a very low risk to mammals. 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. The results of a single study show that disparlure is not mutagenic.

3.1.2 Mechanism of Action

As discussed in Section 4.1.2.3, the mechanism of action for the efficacy of disparlure as an attractant for male gypsy moths has been well characterized. However, since disparlure has very low toxicity to mammals, studies on the mechanism of action for toxicity of disparlure in mammals have not been conducted. Thus, there is no information available in the FIFRA files or in the open literature regarding the mechanism of toxicity (if any) of disparlure in mammals.

3.1.3 Kinetics and Metabolism

No studies designed specifically to obtain information on the kinetics or metabolism of disparlure were identified. The kinetics of absorption of disparlure following dermal, oral or inhalation exposure are not documented in the available literature. Disparlure appears to persist in humans for long periods of time. This supposition is based on a case report of an individual who had direct dermal contact with disparlure in 1977 (Cameron 1981, 1983, 1995). This individual appears to have attracted male gypsy moths for a period of over 15 years. It is estimated that the exposure level of this individual to disparlure was very low, although no quantitative estimates of exposure were reported.

Assays have been conducted using disparlure and several natural and xenobiotic epoxides to determine the ability of each to induce epoxide metabolizing enzymes (Moody et al. 1991). Male mice were given 500 mg a.i./kg/day disparlure by intraperitoneal injection for 3 days. This was the maximum dose tested in preliminary range finding studies. Exposure to the compound had no effect on relative liver weight, using matched controls, or microsomal protein. Relative cytosolic protein was significantly ($p<0.05$) increased by 18% over control values. Disparlure also caused a moderate but statistically significant ($p<0.05$) increase in microsomal cholesterol epoxide hydrolase activity. This study suggests that very high doses of disparlure may induce enzymes involved in the metabolism of disparlure. Given the very low levels of exposure to disparlure that are likely in the use of this agent in gypsy moth control programs, this study has no direct relevance to this risk assessment.

3.1.4 Acute Oral Toxicity

Other than standard bioassays for acute toxicity that were conducted as part of the registration process, no information regarding the acute toxicity of disparlure was identified. The most common measure of acute oral toxicity is the LD_{50}, the estimate of a dose that causes 50% mortality in the test species. As summarized in Appendix 1, there are two studies investigating the acute oral toxicity of high doses of disparlure in rats (Coleman 2000; Kretchmar 1972). Acute oral exposure to 10,250–34,600 mg a.i./kg body weight was not lethal to rats (LD_{50} greater than 34,600 mg a.i./kg) (Kretchmar 1972). Disparlure was administered, undiluted, by gavage, and the rats were observed for 14 days following exposure. This report does not specify whether the test material used was the 50:50 racemic mix or the (+)enantiomer. Necropsy revealed no pathological alterations in any of the treated rats. At all dose levels, however, the animals exhibited hypoactivity, ruffed fur, and diuresis. The significance of these observations cannot be assessed because no control group was used. The apparent NOAEL for mortality and serious clinical toxicity is 34,600 mg a.i./kg, the highest dose tested.

In a more recent study in which rats were administered 5000 mg a.i./kg of a racemic preparation of disparlure, no deaths or pathological abnormalities were observed (Coleman 2000). Clinical signs of toxicity, including piloerection, hunched posture and ungroomed appearance were observed during the first three days following exposure; however, no clinical signs of toxicity were noted during the remaining 11 days of the observation period. As in the study by Kretchmar (1972), no control group was used in the Coleman (2000) study. In this study the LC_{50} is > 5000 mg a.i./kg and the NOAEL is 5000 mg a.i./kg. Thus, with the acute oral LD_{50} exceeding 5,000mg a.i./kg, disparlure would be classified as practically non-toxic using the scheme adopted by U.S. EPA (2003).

3.1.5 Subchronic and Chronic Systemic Toxic Effects

No studies investigating the subchronic or chronic effects of disparlure in mammals were identified. As discussed in Section 8.1.1, studies investigating subchronic and chronic exposures were not required for registration of disparlure (Jacobson 1976; U.S. EPA 1994).

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* is critical because it distinguishes agents that act directly on the nervous system *(direct neurotoxicants*) from those agents that might produce neurologic effects that are secondary to other forms of toxicity (*indirect neurotoxicants*). Virtually any chemical will cause signs of neurotoxicity in severely poisoned animals and thus can be classified as an indirect neurotoxicant.

By this definition, disparlure may be classified as an indirect neurotoxicant. As noted in Section 3.1.4, hypoactivity and piloerection were observed following acute oral exposure to very high doses of disparlure (Coleman 2000; Kretchmar 1972). These observations, however, do not implicate disparlure as a direct neurotoxicant. No studies designed specifically to detect impairments in motor, sensory, or cognitive functions in animals or humans exposed to disparlure were identified. No evidence for disparlure producing direct effects on the nervous system was found.

3.1.7 Effects on Immune System

No studies investigating the effects of disparlure on immune system function in mammals were identified.

3.1.8 Effects on Endocrine System

No studies investigating the effects of disparlure on endocrine system function in mammals were identified.

3.1.9. Reproductive and Teratogenic Effects

No studies investigating the reproductive or teratogenic effects of disparlure in mammals were identified.

3.1.10. Carcinogenicity and Mutagenicity

No studies investigating the carcinogenic activity of disparlure in mammals were identified. A single study investigated the mutagenicity of disparlure with and without metabolic activation in *Salmonella typhimurium* and *Esherichia coli* (Oguma 1998). There was no evidence of mutagenic activity under any of the experimental conditions of this study. This report does not specify whether the test material used was the 50:50 racemic mix or the (+)enantiomer.

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

The primary skin irritation of disparlure was evaluated in a single study using young albino New Zealand rabbits (Kretchmar 1972). Details are provided in Appendix 1. The test sites, located lateral to the midline of the shaved back, were approximately 10 cm apart from one another, and one site was abraded while the other remained intact. The sites were occluded with gauze patches for the duration of the 24-hour exposure period, after which the intact and abraded test sites were examined. The sites were examined and scored again after 72 hours. Signs of mild skin irritation, including erythema and edema, were noted at 24 and 72 hours after application of disparlure. Based on the results of this single study, dermal exposure to a high dose of disparlure appears only mildly irritating to skin and is not a primary skin irritant.

Eye irritation was assayed in a single study in six young New Zealand rabbits exposed to 0.1 mL disparlure (Kretchmar 1972). Details of this study are provided in Appendix 1. Disparlure was instilled into the right eye of each rabbit (the left eye served as a control) to determine the extent of irritation or damage to cornea, iris, and conjunctiva. The severity of ocular lesions was monitored at intervals of 24, 48, and 72 hours. Three of the six rabbits had redness of the conjunctiva at 24 hours, but no effects were observed in any of the rabbits at the later observation periods. No effects were observed 7 days after exposure. Based on the results of this study, disparlure would be classified as a non-irritant for eyes using the scheme proposed by U.S. EPA (2003).

3.1.12. Systemic Toxic Effects from Dermal Exposure

The acute dermal toxicity of disparlure was tested using four young adult New Zealand rabbits (Kretchmar 1972). Study details are provided in Appendix 1. When applied, undiluted, to the shaved backs of the rabbits, 2,025 mg a.i./kg caused local skin reactions after 24 hours of contact with the epidermis. No other dose levels were tested. The rabbits were observed for 14 days after exposure, and the effects observed during this period included dryness (escharosis), skin flaking (desquamation), hemorrhaging, and fissures after 7 days and desquamation, fissures, and pustules after 14 days. Necropsy revealed no pathological alterations other than the effects on the skin. None of the rabbits died as a result of treatment (dermal LD_{50} greater than 2,025mg a.i./kg).

3.1.13. Inhalation Exposure

The acute toxicity of inhalation exposure to disparlure was assessed in rats (Grapenthien 1972). Study details are provided in Appendix 1. Rats were exposed to an aerosol of disparlure for 1 hour, with a calculated average concentration of the aerosol was 5.0 mg a.i./L air. The rats were observed for 14 days after exposure. None of the rats died as a result of exposure. No clinical signs of toxicity were reported. The LC_{50} for inhalation exposure is > 5.0 mg a.i./L air.

3.1.14. Inerts and Adjuvants

As discussed in Section 2, disparlure is typically applied in a slow release polyvinyl chloride formulation and various formulations have been tested and used in USDA programs. As also discussed in Section 2, the USDA uses Disrupt II, a formulation of polyvinyl chloride flakes.

The precise composition of the flake formulation is considered proprietary by Hercon. In the preparation of the current risk assessment, the product manager at Hercon for Disrupt II was contacted and some information on the inerts has been disclosed. The new formulation of Disrupt II contains 5 inert ingredients. Two of the inerts, one of which is identified as diatomaceous earth, are on the U.S. EPA List 4A list and another is on List 4B. A new inert is listed on the exemptions from requiring tolerances 40 CFR 180.910 and 180.930. Polyvinylchloride itself is exempt from tolerance under 40 CFR 180.960 (MacLean 2006).

The reference to the U.S. EPA *List 4* refers to the U.S. EPA method for classifying inert ingredients that are used in pesticide formulations. U.S. EPA classifies inerts into four lists based on the available toxicity information: toxic (List 1), potentially toxic (List 2),

unclassifiable (List 3), and non-toxic (List 4). These lists as well as other updated information on pesticide inerts are maintained by the U.S. EPA at the following web site: http://www.epa.gov/opprd001/inerts/. Any compound classified by U.S. EPA as toxic or potentially toxic must be identified on the product label if the compound is present at a level of 1% or greater in the formulation. If the compounds are not classified toxic, all information on the inert ingredients in pesticide formulations is considered proprietary under Section 10(a) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). In that case, the formulators of the pesticide need not and typically do not disclose the identity of the inert or adjuvant. List 4A is classified as minimal risk inert ingredients. List 4B is defined by the U.S. EPA as follows:

> Other ingredients for which EPA has sufficient information to reasonably conclude that the current use pattern in pesticide products will not adversely affect public health or the environment (http://www.epa.gov/opprd001/inerts/lists.html)

As discussed further in Section 4.1.3.3, some information is available on the toxicity of disparlure, the Disrupt II formulation of disparlure, and Disrupt II flakes that contain only the PVC flakes and other inerts (i.e., no disparlure). While limited, this information suggests that the PVC flakes and other inerts do not contribute to the toxicity of Disrupt II.

3.1.15. Impurities and Metabolites

3.1.15.1. Impurities –Virtually no chemical synthesis yields a totally pure product. Technical grade disparlure does contain low concentrations of four compounds that are structurally related to disparlure – i.e., three octadecenes (all at less than 1%) and one octadecyne (at less than 0.5%) (MTM Chemicals 1991). Additional data regarding impurities in disparlure have been identified in the FIFRA/CBI files (Shin-Etsu Chemical Company 2002; Oguma 2000). The specific information contained in these files is protected under FIFRA Section 12(a)(2)(D) and this information cannot be disclosed in this risk assessment. Nonetheless, concern for impurities is reduced by the fact that the toxicity of impurities should be encompassed in the acute toxicity studies conducted on technical grade disparlure – i.e., disparlure that contains these impurities.

3.1.15.2. Metabolites – No studies on the metabolism of disparlure in mammals were identified in the open literature or the FIFRA/CBI files. Acute toxicity studies, however, typically involve a single exposure followed by a period of observation, most often a 14-day post-dosing period (e.g., U.S. EPA/OPPTS 2003). Because of this, the effects of metabolites formed during the observation period should be encompassed in the acute toxicity studies conducted on disparlure.

3.1.16. Toxicological Interactions.

DDVP pest strips (Vaportape II strip) are contained in the milk carton trap together with a carrier containing disparlure. These milk carton traps are placed in selected areas to monitor gypsy moth infestations. No published literature or information in the FIFRA files permit an assessment of potential toxicological interactions between disparlure and DDVP or any other compounds. A separate risk assessment on DDVP has been prepared as part of the series of risk assessments on the control/eradication agents used for the gypsy moth.

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview

For both workers and the general public, exposures to disparlure may involve multiple routes of exposure (i.e., oral, dermal, and inhalation). Because of the limited toxicity data on disparlure – i.e., no chronic toxicity data are available – no chronic exposure scenarios are developed.

3.2.2. Dermal Exposure

Dermal exposure is most likely to be the predominant route for occupational exposure to disparlure and is also a possible route of exposure for the general public. As discussed in Section 3.1.3, a case report of an accidental exposure of a worker to disparlure shows that no signs of toxicity developed; the only notable effect of disparlure exposure in this worker was the persistent attraction of gypsy moths (Cameron 1981, 1983, 1995). Exposure of this worker was most likely by the dermal route, although the possibility of inhalation exposure cannot be ruled out (Cameron 1995). Since the systemic toxicity of disparlure in mammals is very low, the absence of dermal absorption data does not add significant uncertainty to this risk assessment since no systemic toxicity would be expected to occur, even at very high exposure levels of disparlure. While dermal exposure of workers is expected to be non-toxic, dermal exposure is likely to cause the persistent attraction of gypsy moths.

3.2.3. Inhalation Exposure

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. In these studies, high application rates, relative to the projected rates used in program activities (29.1 g/acre, Section 2.3), were used in order to be able to detect disparlure in air.

Caro et al. (1981) investigated the distribution and persistence of three disparlure formulations including gelatin microcapsules, laminated plastic flakes, and hollow fibers. Each formulation was applied at a rate of 500 g a.i./hectare (approximately 0.45 lb a.i./acre). Release of disparlure from these formulations was most rapid during the first 2 days after application. Initially, air concentrations ranged from approximately 22 to 30 ng/m^3 (nanograms per meter cubed) for microcapsules and fibers and from 7.3 to 8.2 ng/m^3 for flakes. Other investigators using the same application rate reported similar initial concentrations of disparlure in air, approximately 28-30 ng/m^3 for gelatin microcapsules and laminated plastic flakes (Taylor 1982). At a lower application rate (250 g/hectare), there were somewhat higher levels, 44.5-99.3 ng/m^3, using gelatin microcapsules (Plimmer et al. 1977).

Over time, the concentrations of disparlure in air will decrease as the disparlure dissipates. After 30 days, air concentrations ranged from approximately 0.4 to 2.5 ng/m^3 for all formulations (Caro et al. 1981). Flakes that originally contained 7.1% disparlure (w/w) contained 6.0% (w/w) disparlure (85% of the original level) by 30 days after treatment. Results of a study using a disparlure gelatin microcapsule formulation show that release rates increase with increasing temperature (Caro et al. 1977).

The highest reported air concentration after aerial application of 250 g/hectare racemic disparlure on flakes is slightly less than 100 ng/m^3 (Taylor 1982). At an application rate of nearly 30 g/acre, concentrations of approximately 30 ng/m^3 can be expected. Since this estimate is based on the highest levels of disparlure in air, which occur within the first 5 days after application (Caro et al. 1981, Taylor 1982), actual levels of exposure could be lower.

Air concentrations resulting from the release of disparlure from traps are expected to be low relative to air concentrations resulting from aerial application of disparlure. Traps contain only 0.5 mg disparlure/trap. The rate of dissipation of disparlure from traps is dependent upon many factors, including dispenser design, lure type, and air temperature and flow (Bierl 1977, Bierl-Leonbardt 1979, Leonhardt et al. 1990). Thus, air concentrations resulting from volatilization of disparlure from traps are expected to be very low and highly variable.

Over a 120-day period, 38 to 68% of disparlure was lost from lures in laminated plastic dispensers, with loss varying over a variety of experimental conditions (Bierl-Leonbardt 1979). Loss of (+)disparlure was reduced with the use of thicker plastic dispensers and increased with increasing air flow rate and increasing temperature. Greenhouse studies have shown that approximately 50%–80% of (+)disparlure is released from PVC twine or laminates during a 16-week aging process (Kolodny-Hirsch and Webb 1993). Release rates 30 to 40 ng/hr were noted from cotton wicks containing 100 μg (+)disparlure, with increased rates observed at higher temperatures.

3.2.4. Oral Exposure

Although the efficacy of disparlure depends on its volatility, the studies summarized above demonstrate that 70%–85% of disparlure may remain in the carrier matrix after prolonged periods of time. Consequently, oral exposure may occur from consumption of disparlure flakes or tape. At an application rate of approximately 30 g/acre, an individual would have to consume all of the flakes in a 1 m^2 area to receive a dose of 7.4 mg. If this were done by a 10 kg child, the dose would be 0.74 mg/kg.

3.3. DOSE-RESPONSE ASSESSMENT

The toxicity data on disparlure are not adequate for making a standard dose-response assessment. As detailed in Section 3.1, the limited available data indicate that disparlure has a low order of acute toxicity, based on mortality as the endpoint:

Oral LD_{50} >34,600 mg/kg
Dermal LD_{50} >2,025 mg/kg
Inhalation LC_{50} >5 mg/L x 1 hour

Data regarding the toxicity of disparlure to animals or humans after subchronic or chronic exposures were not located in the available literature. Moreover, the acute toxicity of this compound for endpoints other than mortality is poorly characterized.

3.4. RISK CHARACTERIZATION

3.4.1 Overview

Although studies on the acute toxicity of disparlure have been conducted in laboratory animals, the lack of subchronic or chronic toxicity data precludes a quantitative assessment of risk for longer-term exposures. 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 that cannot be quantified. Other uncertainties in this analysis are associated with the exposure assessment and involve environmental transport and dermal absorption. 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 low doses of disparlure used in programs to control the gypsy moth.

3.4.2. Workers and the General Public

It is not possible to develop a reference dose (RfD); therefore, the calculation of a hazard quotient (level of exposure divided by the RfD) and a standard risk characterization cannot be developed. Nonetheless, the limited information that is available regarding the use and toxicity of disparlure gives no clear indication of hazard. For example, the plausible level of oral exposure to a small child is less than 1 mg/kg (Section 3.1.4). This is a factor of 10,000–35,000 less than the exposure levels that were not lethal to rats (Kretchmar 1972, Section 3.1.4). Empirical relationships between acute exposure levels that are lethal to experimental mammals and subchronic or chronic NOAELs in experimental mammals (for example, Dourson and Stara, 1983) do not suggest that the use of disparlure to control of the gypsy moth is likely to pose a substantial hazard to humans.

The only clear and unequivocal biological activity of disparlure is its ability to attract the male gypsy moth. Because disparlure appears to be highly persistent in humans, dermal contact with the compound might make an individual an attractant to male moths over a period of many years. Although this is not likely to cause adverse health effects, it is likely to be a nuisance.

3.4.3. Sensitive Subgroups

The toxic effects of disparlure, if any, have not been identified. Consequently, groups at special risk, if any, cannot be characterized. Because disparlure attracts the male gypsy moth, individuals who have an aversion to insects might be considered to be a sensitive subgroup. Nonetheless, this aversion and sensitivity would not be related to any frank health effect.

3.4.4. Cumulative Effects

Very little information is available on the toxicity of disparlure. As noted above, the ability to attract the male gypsy moth is the only clear biological activity of this compound. Since this compound seems to persist in humans for prolonged periods, repeated exposures are more likely than single exposures to transfer sufficient quantities of disparlure to the individual to attract the moth.

3.4.5. Connected Actions

No information is available on the interaction of disparlure with other control agents or other chemicals usually found in the environment. There is an obvious and substantial interaction of disparlure with the adult male gypsy moth. Individuals who are exposed to sufficient quantities of disparlure and who live in an area in which male gypsy moths reside will attract the moth. The definition of a sufficient quantity of disparlure, however, cannot be characterized from the available data.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview

There is very little information regarding the toxicity of disparlure to nontarget wildlife species. As discussed in Section 3.1, rigorous toxicity testing of disparlure was not required by the U.S. EPA (U.S. EPA 1994). Thus, the only studies identified in the available literature are acute toxicity studies in bobwhite quail, mallard ducks, rainbow trout, bluegill sunfish, *Daphnia magna* and Eastern oysters. No chronic toxicity studies were identified in the available literature.

Results of acute gavage and dietary toxicity 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 bw in bobwhite quail.

Limited data are available regarding the toxicity of disparlure to aquatic animals. A major issue in the interpretation of the aquatic toxicity data on disparlure involves the solubility of disparlure in water. While no measured values for the solubility of disparlure in water are available, estimates based on quantitative structure-activity relationships developed by the U.S. EPA suggest that the solubility of disparlure in water is in the range of 0.0019 to 0.0028 mg/L. The bioassays that have been conducted on disparlure and Disrupt II formulations of disparlure have not measured concentrations of disparlure in the test water but report nominal concentrations of disparlure that exceed the water solubility of disparlure by factors of about 10 [0.028 mg/L] to over 150,000 [300 mg/L]. Based on the results of the available bioassays and considerations of water solubility, disparlure does not appear to present any toxic hazards to aquatic species. In toxicity tests of small aquatic invertebrates (i.e., daphnids), trapping of the organism at the surface of the water has been noted in bioassays and this can present a physical hazard to the organism. The significance of this physical hazard observed in bioassays to potential hazards in field applications is unclear.

4.1.2. Toxicity to Terrestrial Organisms

4.1.2.1. Mammals– As discussed in Section 3.1, there is very little information on the toxicity of disparlure in mammalian species. Results of acute toxicity studies for oral, dermal, ocular and inhalation exposure to disparlure show that disparlure has very low toxicity to mammals. Other than some minor clinical signs of toxicity (i.e., piloerection, hunched posture and ungroomed appearance in rats), acute oral exposure of rats to very high doses of disparlure (up to 34,600 mg a.i./kg bw) did not result in death or signs of systemic toxicity in rats (Kretchmar 1972). Thus, acute exposure to disparlure does not appear to exhibit any organ-specific toxicity. There is no information available regarding the effects of chronic exposure of mammals to disparlure. No field studies are available in which the impact of disparlure were assessed on mammalian wildlife communities.

4.1.2.2. Birds– As summarized in Appendix 2, the acute toxicity of disparlure administered by gavage has been studied in bobwhite quail (Fink et al. 1980) and acute exposure to dietary disparlure has been studied in bobwhite quail chicks and mallard ducklings (Hudson 1975). In adult bobwhite quail administered single doses of disparlure ranging from 398 to 2510 mg a.i./kg by gavage, no mortalities were observed at any dose level (Fink et al. 1980). In the highest dose group, lethargy was observed in 3 of 10 birds; it is unclear if this observation was treatment related. In quail chick and mallard ducklings exposed to 313 to 5000 ppm disparlure in the diet for 5 days, no mortalities were observed and no clinical signs of toxicity were reported during the 14-day observation period. Based on the results of these studies, the LD_{50} for a single dose of disparlure administered by gavage to bobwhite quail is > 2510 mg a.i./kg bw and the corresponding value for 5-day dietary exposure to quail chicks and mallard ducklings is > 5000 ppm.

4.1.2.3. Terrestrial Invertebrates– As discussed in Section 2, disparlure is a naturally occurring insect pheromone. The mechanism of action of disparlure in disrupting gypsy moth mating is well characterized. The (+)disparlure enantiomer, which is produced and released by female gypsy moths, is a powerful attractant to male gypsy moths. Male gypsy moths detect disparlure through highly specific detectors located on antennae (Murlis et al. 2000, Plettner et al. 2000). The (–)disparlure enantiomer is a receptor antagonist to (+)disparlure and has slight repellent activity (Plettner et al. 2000). When sprayed over a large area, disparlure disrupts mating by confusing male moths. There are a large number of greenhouse and field studies showing that disparlure is an effective agent in decreasing gypsy moth populations (Beroza et al, 1975, Campbell 1983, Herculite Products Inc., 1978, Kolodny-Hirsch and Webb 1993, Leonhardt et al. 1990, Leonhardt et al. 1993, Leonhardt et al. 1996, Plimmer et al. 1977, Schwalbe et al. 1978, Schwalbe et al. 1979, Sharov et al. 2002, Thorpe et al. 1993, US Department of Agriculture 1973).

Although disparlure is considered highly selective for gypsy moths, there is some evidence showing that disparlure may have effects on the mating of other species of moths. As part of the reproductive communication between male and female nun moths, female nun moths produce a blend of pheromones that contains disparlure (Gries et al. 2001). Studies show that lures containing disparlure are effective in attracting male nun moths (Gries et al. 2001, Morewood et al. 1999, Morewood et al. 1999). The potency of disparlure in attracting male gypsy moths relative to nun moths has not been assessed. Disparlure is also produced by *L. fumida* [a species native to Japan] (Schaefer et al. 1999). Thus, based on the results of these studies, it appears that disparlure is not completely selective for the gypsy moth. Although studies have not been conducted, it is possible that other closely related species of moths could also respond to disparlure.

No laboratory or field studies on the effects of acute or chronic exposure of disparlure to other terrestrial invertebrates were identified in the available literature.

4.1.2.4. Terrestrial Plants (Macrophytes)–Neither the published literature nor the U.S. EPA files include data regarding the toxicity of disparlure to terrestrial plants.

4.1.2.5. Terrestrial Microorganisms– Neither the published literature nor the U.S. EPA files include data regarding the toxicity of disparlure to terrestrial microorganisms.

4.1.3. Aquatic Organisms

4.1.3.1. Fish – As summarized in Appendix 3, acute toxicity studies of disparlure were conducted in rainbow trout and bluegill sunfish (Knapp and Terrell 1980, Rausina no date). No effect on survival was observed in bluegill sunfish exposed to disparlure at a nominal concentration of 100 mg/L (Rausina no date) or 300 mg/L (Knapp and Terrell 1980) for up to 96 hours. The 96-hour LC_{50} for bluegill sunfish is >300 mg/L. In rainbow trout, no effect on survival was observed following exposure to 100 mg/L disparlure for 48 hours (Rausina no date). However, after 72 hours of exposure to 100 mg/L disparlure, only 8 of 10 trout survived. Survival of trout was not affected at disparlure concentrations of 0.1 to 10 mg/L. Under these experimental conditions, the NOEC for mortality in rainbow trout is 10 mg/L.

Neither of these studies would be considered acceptable by current standards for toxicity studies in fish (e.g., U.S. EPA/OPPTS 2006). For example, the U.S. EPA guidelines for acute toxicity studies in fish require information on the solubility of test compound in water and require that the test substance not be tested as concentrations in excess of the solubility of the compound in water.

As noted above and detailed further in Appendix 3, neither Rausina (no date) nor Knapp and Terrell (1980) measured the concentration of disparlure in the test water. As noted in Section 2, no measured values are available for the solubility of the disparlure in water. Based on quantitative structure activity relationships (QSAR), however, it is likely that the solubility of disparlure in water is very low. As indicated in Table 2-1, the QSAR package developed by the U.S. EPA estimates a water solubility for disparlure of 0.0019 to 0.0028 mg/L (EPI Suite 2006). In the preparation of this risk assessment, Hercon (the company that manufactures the Disrupt II flakes) was contacted and the chemists at Hercon indicated that they were not aware of any measured water solubility values for disparlure but, consistent with the estimates from EPI Suite (2006), the chemists at Hercon indicated that the water solubility is likely to be very low.

The importance of considering water solubility in the assessment of a chemicals toxicity to aquatic species is discussed by Clements et al. (1996), the individuals who developed the toxic estimation algorithms used in EPI Suite. Essentially, if a compound is non-toxic at the limit of water solubility, then the compound can be classified as presenting no plausible toxic risk to the organism. Physical hazards may still be plausible. This is discussed further in Section 4.1.3.3 (Aquatic Invertebrates).

The toxicity values estimated by EPI Suite (2006) using algorithms of Clements et al. (1996) are summarized in Table 4-2. The algorithms used to estimate the toxicity values were developed by Clements et al. (1996) and are based on regression equations which take the general form of:

$$Log_{10}(TV) = m\ Log_{10}(K_{ow}) + b$$

where *TV* is the toxicity value in units of millimoles/liter (mM/L), Kow is the octanol/water partition coefficient, and *m* and *b* are model parameters (slope and intercept, respectively). While the algorithms are based on molar concentrations, EPI Suite converts these concentrations to units of mg/L for the output files. The specific model parameters are summarized in Table 4-2 and are based on QSAR estimates for mono-epoxides – i.e., compounds structurally similar to disparlure.

A very important feature of these estimates concerns the limiting values for the Kow of the compound. As discussed by Clements et al. (1996), this recommended limiting value is based on the range of Kow values on which the QSAR estimates are based. For mono-epoxides, the limit recommended by Clements et al. (1996) is 5. As noted in Table 2-1, the estimated log Kow value for disparlure is 8.08 – i.e., higher than the recommended cut off value by a factor of about 1000.

This cutoff value is very important in the interpretation of the estimated toxicity values. As indicated in Table 4-2, the estimated toxicity values for fish range from about 0.12 to 0.14 mg/L based on the Kow. Although the studies by Knapp and Terrell (1980) as well as Rausina (no date) have serious limitations, they clearly indicate no mortality at the nominal concentrations. It is likely, however, that the actual concentrations would not have exceeded the water solubility of disparlure – i.e., 0.0019 to 0.0028 mg/L (Table 2-1). The simple interpretation is that the water solubility of disparlure is so low that the maximum possible concentration in water is below the estimated toxicity values by a factor of about 43 [0.12 mg/L ÷ 0.0028 mg/L] to 74 [0.14 mg/L ÷ 0.0019 mg/L]. This is the basis for asserting that disparlure is not likely to pose a risk of toxicity to fish.

Thwaits and Sorensen (2005) have recently submitted a brief summary of a study using rainbow trout in which disparlure was assayed for olfactory stimulation. At nominal concentrations of either 0.028 mg/L or 0.28 mg/L, with or without the presence of methanol (used to enhance the solubility of disparlure in water), disparlure evidenced no activity relative to negative controls (well water or well water with methanol) or L-serine as a positive control.

4.1.3.2. Amphibians– Neither the published literature nor the U.S. EPA files include data regarding the toxicity of disparlure to amphibian species.

4.1.3.3. Aquatic Invertebrates – As with fish, the data on the toxicity of disparlure itself to aquatic invertebrates is relatively old (LeBlanc et al. 1980; Ward 1981) and these studies would not meet the current requirements of the U.S. EPA (e.g., U.S. EPA/OPPTS 2006) because of the same limitations discussed in Section 4.1.3.1 (Fish). The acute toxicity of disparlure to *Daphnia* was evaluated in a single study (LeBlanc et al. 1980). Details of this study are provided in Appendix 3. A dose-related increase in mortality was observed following 48 hours of exposure, with 7% mortality at 0.028 mg/L and 100% mortality at a 0.22 mg/L. The LC_{50} value was calculated at 0.098 mg/L and the NOEC for mortality was 0.017 mg/L. In Eastern oysters exposed to 1.25 to 20 mg/L disparlure for 96 hours, there was no effect on new shell growth (Ward 1981). Again, all of these toxicity values refer to nominal concentrations rather than

measured concentrations and all of these toxicity values exceed the plausible range of the solubility of disparlure in water – i.e., 0.0019 to 0.0028 mg/L (Table 2-1).

The major difference, however, between the data on fish and data on daphnids involves the mortality. As detailed in Appendix 3, LeBlanc et al. (1980) report a clear dose-response relationship for daphnids. The important detail, however, is that this mortality was associated with organisms being trapped at the air-water interface. While not discussed by LeBlanc et al. (1980), it is likely that the entrapment of the daphnids at the air-water interface was attributable to the undissolved disparlure in the test solution. Based on the highest estimate of the solubility of disparlure in water (i.e., 0.0028 mg/L) the nominal test concentrations used by LeBlanc et al. (1980) exceed the solubility of disparlure in water by factors of 10 [0.028 mg/L ÷ 0.0028 mg/L] to about 78 [0.22 mg/L ÷ 0.0028 mg/L].

The supposition that daphnid mortality in the study by LeBlanc et al. (1980) is due to the physical trapping of the organisms at the water surface by undissolved disparlure is supported by the more recent studies by Palmer and Krueger (2006a,b) on various formulations of Disrupt II flakes. The studies were sponsored by the Forest Service because of concerns with the quality of the data on disparlure, the preliminary risk assessment on disparlure (SERA 2004), as well as a desire to better characterize the potential hazards of the inerts used in Disrupt II formulations.

The studies by Palmer and Krueger (2006a,b) involved Disrupt II formulations that were designated as *Standard Flakes* and *Modified Flakes*. This nomenclature is somewhat awkward but will be maintained because these terms are used in the reports by Palmer and Krueger (2006a,b) and these terms are also used (at least currently) by individuals in the USDA who are involved in applications of Disrupt II (e.g., Leonard 2006b). *Standard flakes* refer to an older formulation that was the only formulation used operationally in USDA programs up through 2003. Hercon modified their Disrupt II formulation by changing one of the inert ingredients and these modified flakes were first tested by USDA in 2002. By 2004 the modified formulation of Disrupt II had replaced the standard formulation in most operational applications (Leonard 2006d). As noted in Section 2, the USDA has been involved in the refinement of various formulations of disparlure for many years and it seems likely that new formulations will be developed in the future.

Standard Flakes were tested in the study by Palmer and Krueger (2006a) and *Modified Flakes* were tested in the study by Palmer and Krueger (2006b). Both of these studies involved identical experimental designs, the details of which are given in Appendix 3. Both studies involved three set of flakes: blank flakes that contained no disparlure (i.e., only the inerts), fully formulated flakes that were manufactured in 2003, and fully formulated flakes that were manufactured in 2005.

In each study, the daphnids were exposed to a series of six water accommodated fractions (WAF) at nominal concentrations of 0.18, 0.54, 1.8, 5.4, 18, and 54 mg a.i./L. The technique using water accommodated fractions is a method specifically designed for water insoluble compounds (e.g., French-McCay 2002; Pelletier et al. 1997). As implemented by Palmer and Krueger (2006a,b), the application of this method involved mixing the flakes (formulated or

blank) into 12 L of dilution water and stirring the mixture for approximately 24 hours. The test water (without flakes) was then decanted into the test chambers into which the daphnids were placed.

As with the studies in fish and the earlier studies with invertebrates, the concentration of disparlure in the test water was not measured. Consequently, the "concentrations" of disparlure are reported as *nominal concentrations* rather than *measured concentrations*. As detailed in U.S. EPA guidelines for the conduct of acute bioassays in *Daphnia* (U.S. EPA 1996), the U.S. EPA guidelines for toxicity studies in *Daphnia* require measurements of the concentrations of the test substance in water. The rationale for this requirement is simple: if the concentration is not measured, there may be substantial uncertainty in attempting to characterize the exposure. The distinction between *nominal concentrations* and *measured concentrations* is particularly important for compounds such as disparlure which have a very low solubility in water. As detailed further below, the *nominal concentrations* of disparlure in the toxicity studies of disparlure and Disrupt II flakes substantially exceed the water solubility. This leads, in turn, to the development of a film on the surface of the water and this film traps the daphnids. Thus, the effect, while adverse, appears to be a physical rather than toxic effect.

As detailed in Appendix 3, the blank flakes – i.e., the flakes without disparlure – did not result in any mortality in any of the test groups for either the *Standard Flakes* (Palmer and Krueger 2006a) or the *Modified Flakes* (Palmer and Krueger 2006b). The flakes from 2003 – both standard and modified – resulted in very low rates of mortality and immobility and the estimated LC_{50} values in both of these bioassays were >300 mg formulation/L, equivalent to >53 mg a.i./L.

The new flakes from 2005 – again both standard and modified – yielded much lower estimates of the 48 hour-LC_{50}: 69 mg formulation/ L (12.3 mg a.i./L) for standard flakes (Palmer and Krueger 2006a) and 48 mg formulation/L (8.6 mg a.i./L) for modified flakes (Palmer and Krueger 2006b). The reason or reasons for the differences between the 2003 flakes and the 2005 flakes is unclear and this issue is not addressed in the report by Palmer and Krueger (2006a,b) other than to note the differences in toxicities. For the standard flakes, Palmer and Krueger (2006a) note only the following differences in physical appearance:

> *The SF 2003 and SF 2005 test solutions and the blank solution appeared clear and colorless in the test chambers at test initiation. At test termination, all of the solutions, with the exception of the 300 mg/L SF 2005 solution, appeared clear and colorless. The 300 mg/L SF 2005 test solution appeared clear and colorless with white particulates on the bottom of the test chamber.* (Palmer and Krueger (2006a, p. 12.)

For the modified flakes, Palmer and Krueger (2006b) note differences in appearance between the 2003 and 2005 flakes that are somewhat more striking than those for the standard flakes:

> *Prior to decanting, the MF 2003 and MF 2005 WAF solutions, and the blank solution, appeared clear and colorless, with white particles on the surface of the water and green and white particles settled on the bottom of the WAF bottles, increasing in amount with increasing concentration. The MF 2003 and MF 2005 test solutions and the blank solution appeared clear and colorless in the test chambers at test initiation and termination.* (Palmer and Krueger (2006b, p. 12.)

During the period when these bioassays were being conducted, the testing facility was visited by a toxicologist with the USDA Forest Service who reported striking differences in the appearance of the 2003 and 2005 flakes, both standard and modified, prior to mixing the flakes with water (Appleton 2006).

As detailed in Appendix 3, the recent bioassays on the flake formulations using daphnids (Palmer and Krueger 2006a,b) are similar to the earlier bioassay on technical grade disparlure using daphnids (LeBlanc et al. 1980) in that all of these studies observed daphnids trapped at the surface of the water. While LeBlanc et al. (1980) did not report the numbers of daphnids that were trapped at various nominal concentrations, the data reported by Palmer and Krueger (2006a,b) clearly indicate an association between the nominal concentrations, number of organisms trapped at the water surface, and subsequent mortality or immobility.

The observations in these studies and the QSAR estimate of the very low water solubility of disparlure (Table 2-1) suggest that the trapping of the daphnids at the surface of the water was due to a layer of insoluble disparlure at the surface of the test water. Because no daphnids were trapped at the water surface in the bioassays on the blank flakes, both standard and modified, it is not plausible to assert that any of the inerts in either the standard or modified flakes contributed to the entrapment of the organisms at the water surface.

When daphnids are trapped at the surface of the water, the organisms are under substantial stress and, if they remain trapped for a prolonged period, the animals may die for reasons that are not directly related to the systemic toxicity of the disparlure – e.g., impaired respiration. This is noted by Palmer and Krueger (2006a,b) in both sets of bioassays:

> *Due to the nature of the test substance, mortality/immobility among daphnids in the Disrupt II formulation treatment groups may have been due, in part, to a physical effect, rather than only to toxicity.* (Palmer and Krueger (2006a,b p. 15)

As with fish, the weight of the evidence suggest that disparlure will not pose any risk to daphnids in terms of toxicity. Unlike fish, however, the available data clearly indicated that disparlure could pose a physical hazard to daphnids and possibly other aquatic invertebrates if the amount of disparlure in the water is sufficient to create an insoluble film of disparlure on the surface of the water.

While the hazard during a laboratory bioassay is clearly documented, the likelihood of this physical hazard occurring in the field after a normal application of disparlure is more difficult to assess. Disrupt II is not intentionally applied to water. While no microcosm or mesocosm studies have been conducted, Disrupt II as well as other experimental formulations of disparlure have been used by the USDA for over a decade. In that period, no incidents or field observations have been made that would suggest any adverse effects on aquatic invertebrates (Leonard 2006c). The potential for a physical hazard to aquatic invertebrates is considered further in Section 4.4.4 (risk characterization for aquatic invertebrates).

4.1.3.4. Aquatic Plants– Neither the published literature nor the U.S. EPA files include data regarding the toxicity of disparlure to aquatic plants.

4.1.3.5. Other Aquatic Microorganisms– Neither the published literature nor the U.S. EPA files include data regarding the toxicity of disparlure to aquatic microorganisms.

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview

As discussed in Sections 3.1 and 4.1, disparlure appears to be essentially nontoxic to mammals and birds. While this assessment is limited by the lack of chronic toxicity data in terrestrial species, it is not expected that acute or chronic exposure of terrestrial mammals or birds to disparlure would result in the development of significant adverse effects. Given the low toxicity of disparlure and limited available data, an exposure assessment for terrestrial species would not add to the assessment of risk for terrestrial species. Thus, an exposure assessment for terrestrial species is not included in this risk assessment. For aquatic species, the range of plausible nominal concentrations of disparlure in water are calculated at 0.0015 mg/L to 0.0037 mg/L over the range of applications rates considered in this risk assessment – i.e., 6 g a.i./acre to 15 g a.i./acre. These concentrations apply to a 1 meter deep body of water. The lower end of this range is within the estimated solubility of disparlure in water – i.e., 0.0019 to 0.0028 mg/L – and the upper end of this range slightly exceeds the estimated solubility of disparlure in water.

4.2.2. Exposure of Aquatic Animals

Disparlure is not intentionally applied to bodies of water (Hercon 2006a; Leonard 2006b). Thus, under normal conditions, aquatic organisms are not likely to be exposed to substantial amounts of disparlure. Accidental applications to surface water have been reported (Leonard 2006c) and these can be considered.

Disrupt II flakes could be accidentally applied to either standing bodies of water (e.g., ponds or lakes) or moving bodies of water (e.g., streams or rivers). As discussed in Section 4.1.3, there is no basis for asserting that disparlure will pose any risk of toxic effects to aquatic organisms at the limit of estimated solubility of disparlure in water. The only risk that can be identified is the entrapment of small aquatic invertebrates in a surface film of disparlure (Section 4.1.3.3). A surface film of disparlure could occur if Disrupt II flakes were accidentally applied to a standing body of water, such as a lake or pond, in a sufficient amount to exceed the solubility of disparlure in the water. The development of a film in a flowing body of water, such as a stream or river, does not appear to be plausible. Consequently, for this risk assessment, exposure scenarios are developed only for standing bodies of water and these scenarios are used to assess potential effects only on small aquatic invertebrates that might interact with the surface of the water – i.e., benthic species are not considered to be at any risk.

If Disrupt II flakes are applied to a standing body of water, some disparlure will volatilize into the air and some disparlure will leach from the flakes into the water. The disparlure in the water will diffuse through the water and a film of disparlure on the surface of the water will form if the water becomes saturated. The film on the surface of the water with then volatilize over time. The kinetics of these processes cannot be characterized. Nonetheless, the bioassays conducted by Palmer and Krueger (2006a,b) suggest that this general scenario is plausible. Thus, in the exposure assessment for small aquatic invertebrates, instantaneous leaching will be assumed and the impact of volatilization will not be considered. These are conservative assumptions in that

they will tend to overestimate exposure. This is considered further in Section 4.4.4 (risk characterization for aquatic invertebrates).

As discussed in Section 2.3, this risk assessment considers application rates in the range of 6 grams a.i./acre to 15 grams a.i./acre. This range corresponds to application rates of about 1.5 mg/m^2 [6 grams a.i./acre × 1000 mg/g ×1 acre/4047 m^2 = 1.4826 mg/m^2] to 3.7 mg/m^2 [15 grams a.i./acre × 1000 mg/g ×1 acre/4047 m^2 = 3.7064 mg/m^2]. If these amounts of disparlure are applied accidentally to a 1 meter deep body of water, nominal concentrations – i.e., assuming complete mixing and ignoring solubility limitations – would be in the range of 0.0015 mg/L to 0.0037 mg/L [1000 liters per m^3]. Details of these calculations are given in Worksheet A01of the EXCEL workbook that accompanies this risk assessment.

As noted in Table 2-1 and discussed in Section 4.1.3, no measured values for the solubility of disparlure in water are available but estimates based on quantitative structure-activity relationships developed by the U.S. EPA (EPI Suite 2006) suggest that the solubility of disparlure in water is in the range of 0.0019 to 0.0028 mg/L. Thus, the nominal concentrations that might occur in a 1 meter deep body of water after an accidental direct application are within the estimated water solubility of disparlure at the lower bound of the application rate (i.e., an application rate of 6 g a.i./acre) [0.0015 mg/L < 0.0028 mg/L] but modestly exceed the estimates of the solubility of disparlure in water at the upper bound of the application rate by a factor of about 1.3 [0.0037 mg/L ÷ 0.0028 mg/L].

Deeper bodies of water will result in lower concentrations that are likely to be at or below the solubility of disparlure in water and shallower bodies of water would lead to nominal concentrations that would exceed the solubility of disparlure in water. This type of situational variability is difficult to encompass in a general risk assessment. As a tool for individuals who are involved in or wish to assess applications of disparlure under conditions other than those considered in this risk assessment, the workbook that accompanies this risk assessment includes a worksheet (named A02) that can be used to calculate nominal concentrations of disparlure based on specified application rates, fractional deposition (i.e., drift), and average depth of the water body. Worksheet A02 also calculates hazard quotients based on the dose-response assessment for daphnids (Section 4.3.3).

Note that Worksheet A02 applies only to the accidental application of disparlure to a standing body of water. No exposure scenarios are developed for accidents that involve the dumping of large amounts of Disrupt II into a standing body of water. While such accidents are possible, none have been documented. In addition, the calculation of nominal concentrations is trivial under the assumption of instantaneous mixing – i.e., the amount of disparlure that is deposited in the water divided by the volume of the water. Given the available information on the toxicity of disparlure to aquatic species (Section 4.1.3), no further elaboration of this exposure assessment is warranted. Potential consequences for aquatic species are discussed in Section 4.4.3 (risk characterization for fish) and Section 4.4.4 (risk characterization for aquatic invertebrates).

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1 Overview

Given the low toxicity of disparlure to terrestrial animals coupled with the limitations imposed by the lack of chronic toxicity data, no standard dose-response assessment can be made or is warranted for disparlure in terms of effects on terrestrial species. As reviewed in Section 4.1.2.3, disparlure is produced by other species of moths and has the ability to attract nun moths (Gries et al. 2001, Morewood et al. 1999, Morewood et al. 1999, Schaefer et al. 1999). However, since there are no quantitative data available regarding the efficacy of disparlure in nun moths, a dose-response assessment for this effect in a nontarget species cannot be made. Similarly, no explicit dose-response relationship is proposed for fish. There is no basis for asserting that adverse effects in fish are plausible under any foreseeable conditions. For aquatic invertebrates, there is no basis for asserting that toxic effects are likely at the limit of the solubility of disparlure in water. At nominal concentrations that exceed the solubility of disparlure in water, small invertebrates that may interact with the water-surface interface could become trapped in this interface due to a layer of undissolved disparlure at the air-water interface.

4.3.2. Fish

As discussed in Section 4.1.3.1, the available information on the toxicity of disparlure to fish are extremely limited. Nonetheless, there is no basis for asserting that disparlure is likely to pose a risk to fish at the limits of water solubility – i.e., in the range of 0.0019 to 0.0028 mg/L (Table 2-1) – or at nominal concentrations that are substantially in excess of the solubility of disparlure in water. Consequently, no formal dose-response relationship for fish is proposed. Nonetheless, it is noted that a nominal concentration of 10 mg/L from the study by Rausina (no date) is a clear NOEC – see Appendix 3 for details and the discussion in Section 4.1.3.1. This nominal concentration is a factor of about 3,500 to over 5,000 above the estimated values for the concentration of disparlure in water. The implications of this range of values are discussed further in Section 4.4.3.

4.3.3. Aquatic invertebrates

The risk characterization for aquatic invertebrates is somewhat more complicated than that for fish. As with fish, there is no basis for asserting that toxic effects are likely in daphnids at the limit of water solubility. However, as discussed in Section 4.1.3.3, information is available from toxicity tests with daphnids of both technical grade disparlure (LeBlanc et al. 1980) as well as Disrupt II formulations of disparlure (Palmer and Krueger 2006a,b) that exposures to disparlure that exceed the solubility of disparlure in water will result in a film (presumably composed of undissolved disparlure) at the water surface. While this may not pose a toxic risk to daphnids, the toxicity studies demonstrate that these organisms can become trapped at the water surface and this can result in the death of the animal.

The nominal concentrations at which entrapment is pronounced is in the range of the three higher nominal concentrations in the studies by Palmer and Krueger (2006a,b) using the Disrupt II formulations – i.e., a range of about 5.4 mg a.i./L to 54 mg a.i./L. The utility of these values are limited because the amount of disparlure that leached from the flakes used in these bioassays was not determined. On the other hand, these nominal concentrations may better reflect conditions

that could occur in the field – i.e., the processes of leaching from flakes to water as well as volatilization from the water surface to air.

Lower values can be identified from the earlier study by LeBlanc et al. (1980) using technical grade disparlure. As indicated in Appendix 3, the minimum nominal concentration from the LeBlanc et al. (1980) study at which any mortality was noted is 0.028 mg/L. At this concentration, mortality was 1/15. Using the Fischer Exact test (see Section 3.1.5.2. in SERA 2006), this incidence is not statistically significant ($p = 0.5$) and this concentration could be regarded as a NOEC. A similar case could be made for regarding higher concentrations from LeBlanc et al. (1980) as NOEC values: 0.048 mg/L (1/15 mortality, $p = 0.5$) and 0.079 mg/L (2/15 mortality, $p = 0.241379$). The clear LOAEL from the study by LeBlanc et al. (1980) is 0.13 mg/L (12/15 mortality, $p = 0.00000526$). The clear NOEC from this study is 0.01 mg/L at which no mortality was observed. The major limitation in the study by LeBlanc et al. (1980) is that trapping of the daphnids at the water surface is noted but details comparable to those given in Palmer and Krueger (2006a,b) are not provided.

For the current risk assessment, the NOEC value of 0.01 mg/L (nominal concentration) from the study by LeBlanc et al. (1980) will be used for characterizing risk. This is substantially above the estimated water solubility of disparlure – i.e., 0.0019 to 0.0028 mg/L from Table 2-1. As discussed above, the mortality observed in both the study by LeBlanc et al. (1980) as well as the studies by Palmer and Krueger (2006a,b) are probably due to the formation of a slick of disparlure at the surface of the water. Thus, the use of a nominal concentration is simply an index of exposure intended to suggest a slick that would be sufficiently minimal to cause no adverse effect even to small aquatic invertebrates.

No dose-response assessment is proposed for larger aquatic invertebrates or benthic invertebrates. These aquatic invertebrates would not likely be trapped in (large invertebrates) or interact with (benthic species) any slick of disparlure on the surface of the water that might be associated with the application of Disrupt II flakes for the control or eradication of the gypsy moth.

While the studies by Palmer and Krueger (2006a,b) are more recent and contain much more detailed information than is presented in the earlier study by LeBlanc et al. (1980), the Palmer and Krueger (2006a,b) studies are not used explicitly to derive toxicity values. The rationale for this approach is that the study by LeBlanc et al. (1980) does involve the application of known amount of disparlure to the test water. In the studies by Palmer and Krueger (2006a,b), detailed in Section 4.1.3.3, a known amount of Disrupt II flakes was applied to water and a fixed amount of time was allowed for the disparlure to leach from the flakes into the water. The amount of disparlure that actually leached from the flakes into the water, however, was not measured. In addition, the treated water was then decanted to arrive at the test water. The proportion of any leached disparlure that was decanted, however, cannot be determined. Thus, while both the LeBlanc et al. (1980) study and the studies by Palmer and Krueger (2006a,b) involved nominal rather than measured concentrations, the uncertainties in the exposure to disparlure are greater in the studies by Palmer and Krueger (2006a,b). While it may be argued that the Palmer and Krueger (2006a,b) studies might better approximate the impact of an application of Disrupt II

flakes, the Palmer and Krueger (2006a,b) studies did not involve actual exposures to the flakes. Thus, while the Palmer and Krueger (2006a,b) studies were well-designed and provide useful information, the earlier study by LeBlanc et al. (1980) involves fewer uncertainties in terms of the exposure of the daphnids to disparlure.

4.4. RISK CHARACTERIZATION

4.4.1. Overview

As discussed in Section 4.3.1, there is little data available on terrestrial and aquatic animals to allow for a quantitative characterization of risk in species other than rainbow trout and *Daphnia*. Furthermore, the lack of chronic toxicity data in any species adds significant 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 assessed. Nonetheless, given the low toxicity of disparlure based on acute toxicity studies, it is unlikely that exposure to disparlure will result in the development of serious adverse effects in terrestrial and aquatic species. Regarding effects on terrestrial invertebrates, it is not likely that disparlure would disrupt mating of other species of moths that are native to North America (Section 4.1.2.3).

Under normal conditions, aquatic species will not be exposed to substantial levels of disparlure. At the limit of the solubility of disparlure in water, there is no indication that toxic effects are likely in any aquatic species. If Disrupt II flakes are accidently applied over water, the amount of disparlure in the water could result in the formation of an insoluble layer of disparlure at the air-water interface. This would occur only in standing bodies of water (ponds or lakes) and not in flowing bodies of water such as streams or rivers. There is no indication that the formation of disparlure film in a standing body of water would impact fish. Based on toxicity studies conducted in the laboratory, small invertebrates that come into contact with the air-water interface might become trapped in this insoluble film. The likelihood of this occurring and the likelihood of this causing any detectable impact in a body of water is difficult to determine and would vary with the quantity of flakes applied to the body of water and the depth of the body of water. Based on variability in the experimental data as well as the range of application rates used in the USDA programs, hazard quotients would vary from about 0.15 to about 0.37, assuming a 1 meter deep body of water, below the level of concern by factors of about 3 to 10.

4.4.2. Terrestrial Species

Based on the results of acute toxicity studies, the toxicity of disparlure to terrestrial mammals is very low (See Sections 3.1 and 4.1). However, the lack of chronic toxicity studies adds uncertainty to the risk characterization for all terrestrial species. Since results of acute toxicity studies in mammals and birds do not suggest that acute adverse effects are likely, it is not anticipated that exposure of these species to disparlure will results in the development of serious adverse effects in longer term exposures. However, since no chronic toxicity data are available, it is not possible to provide a characterization of risk for longer term exposure.

For terrestrial invertebrates, specifically other species of moths, exposure to disparlure has the potential to disrupt mating. However, due to the lack of data, it is not possible to quantify this risk.

4.4.3. Fish

As discussed in Section 4.1.3.1, the hazard identification for fish indicates that no toxic effects are plausible at the limit of the solubility of disparlure in water. In addition, toxicity studies in fish indicate no effects at nominal concentrations of disparlure in water that factors of about 3,500 to over 5,000 above the estimated values for the concentration of disparlure in water (Section 4.3.2). The reciprocals of these ratios could be taken as approximate hazard indices – i.e., 0.0002 to 0.0003 – and these could be useful in comparing the risks posed by disparlure to risks posed by other agents. A somewhat clearer articulation of the risk characterization, however, is that no risks to fish can be identified under any foreseeable circumstances.

4.4.4. Aquatic Invertebrates

As with fish, there is no indication that disparlure will be toxic to aquatic invertebrates at the limit of the solubility of disparlure in water. Also as with fish, the probability of substantial exposure to disparlure is remote except in the case of accidental misapplication of Disrupt flakes directly to water. Thus, under normal conditions, no risks to aquatic invertebrates can be identified.

The accidental application of Disrupt II flakes to water is plausible and, under some conditions, this could pose risks to aquatic invertebrates that interface with the water surface. This has been clearly demonstrated in laboratory studies with daphnids (Sections 4.1.3.3 and 4.3.3). As discussed in Section 4.2.2, accidental applications to surface water have been reported. If applied to rapidly moving water such as stream, there is no indication that adverse effects would be likely. If applied to standing water, however, concentrations calculated in Section 4.2.2 modestly exceed the estimate of the solubility of disparlure in water at the upper range by a factor of about 3 – i.e., a nominal concentration of 0.0074 mg/L. If the amount of disparlure deposited on the surface of standing water exceeds the solubility of disparlure in water, a surface film could form and some small aquatic invertebrates could be trapped at the air-water interface.

It seems unlikely, however, that this would lead to substantial or even detectable effects based on the clear NOEC value of 0.01 mg/L from the study by LeBlanc et al. (1980). As detailed in Worksheet A01 of the EXCEL workbook that accompanies this risk assessment, the highest calculated hazard quotient is 0.37 and is associated with the application of disparlure at a rate of 15 g a.i./acre to a body of water that is 1 meter deep. The hazard quotient will vary directly with the depth of the water. Since the calculations are based on a 1 meter deep body of water, the hazard quotients would be a factor of 10 lower in a 10 meter deep body of water and a factor of 10 higher in a 0.1 meter deep body of water.

Whether or not the accidental application of disparlure flakes to any body of water would lead to a detectable effect is unclear. As noted in Section 4.1.3.3, no incidents or field observations have been made that would suggest any adverse effects on aquatic invertebrates (Leonard 2006c). However, the only report of an accidental application to water involves application to a river. As noted above, applications to flowing bodies of water would not be expected to result in any adverse effects. Nonetheless, based on the application rates used in vast majority of program activities (Section 2.3), hazard quotients for small aquatic invertebrates would exceed unity only in very shallow bodies of water.

The duration of any exposure to disparlure accidentally applied to water cannot be well characterized. As indicated in Appendix 4, the halftime of disparlure in water is estimated at 360 hours (15 days) based on algorithms used in EPI Suite (Meylan and Howard 2000; U.S. EPA/OPPT 2000). These algorithms, however, rely on estimates of water solubility and Henrys Law constant. As also indicated in Appendix 4, experimental values for the water solubility and Henrys Law constant of disparlure are not available and are themselves estimated by EPI Suite based on molecular structure. This adds uncertainty to the estimated halftime in water. The halftime in water will also be influenced by site-specific conditions as well as the formulation of disparlure in the Disrupt II flakes, increasing the uncertainty in estimates from EPI Suite.

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Table 2-1. Identification and Physical/Chemical Properties of Disparlure.

Property	Value [a]	Reference
CAS Number	029804-22-6	EPI Suite (2006)
Smiles Notation	O(C1CCCCCCCCCC)C1CCCCC(C)C	EPI Suite (2006)
U.S. EPA Registration Number	8730-55	Hercon Environmental, 2004
MW	282.51	EPI Suite (2006)
Henry's Law Constant (atm m^3/mole)	0.015 to 0.061	EPI Suite (2006)
Vapor pressure (mm Hg)	0.00021 to 0.00034	EPI Suite (2006)
Water solubility (mg/L)	0.0019 to 0.0028	EPI Suite (2006)
log $K_{o/w}$	8.08	EPI Suite (2006)
$K_{o/c}$ (acid, ml/g)	3.44×10^4	EPI Suite (2006)
Halftimes in water (days)	0.074 (river) 6.9 (lake)	EPI Suite (2006)
Halftimes in other media (days)	0.5 (air) 15 (water) 30 (soil) 135 (sediment)	EPI Suite (2006)

[a] For many estimates, EPI Suite provides more than one estimate based on different estimation methods. When more than one estimate is provided, the range of values are given. Estimates from EPI Suite are often present out to several decimal places. Except for molecular weight, all values in this table are rounded to two significant places.

Table 2-2: Use of Disparlure by the USDA to control the North American Gypsy Moth from 1995 to 2005 by Type of Use (USDA/FS 2005)

Year	Acres Treated for Eradication	Acres Treated to Slow the Spread
1995	0	2,448
1996	5,352	16,621
1997	0	10,808
1998	7,120	21,418
1999	38,980	19,360
2000	7,988	93,625
2001	0	212,925
2002	650	542,600
2003	0	647,394
2004	250	588,256
2005	0	287,890

Table 3-1: Summary of acute toxicity data of Disparlure in mammals (all values are expressed in terms of a.i.)

Species	Exposure/Dose	Effect	Reference
rat	single oral doses ranging from 10,250 – 34,600 mg/kg	LD_{50} > 34,600 mg/kg NOAEL (mortality) = 34,600 mg/kg	Kretchmar 1972
rat	single oral dose of 5000 mg/kg	LD_{50} > 5,000 mg/kg NOAEL (mortality) = 5,000 mg/kg	Coleman 2000
rat	inhalation exposure, 5.0 mg/L in air for 1 hour	LD_{50} > 5 mg/L air NOAEL (mortality) = 5.0 mg/L air	Grapenthien 1972
rabbit	dermal toxicity testing a single dose of 2,025 mg/kg	LD_{50} > 5,000 mg /kg NOAEL (mortality) = 5,000 mg/kg	Kretchmar 1972
rabbit	primary skin irritation testing a single dose of 0.5 g	Not a skin irritant (only very mild skin irritation)	Kretchmar 1972
rabbit	primary eye irritation testing a single dose of 0.1 g/eye	not an eye irritant	Kretchmar 1972

Table 4-1: Summary of acute toxicity data of Disparlure in avian and aquatic species (all values are expressed in terms of a.i.)

Species	Exposure/Dose	Effect	Reference
bobwhite quail	single oral doses ranging from 398 to 2510 mg/kg (by gavage)	LD_{50} > 2510 mg/kg	Fink et al. 1980
bobwhite quail chicks	313 to 5000 in diet for 5 days	LD_{50} > 5000 ppm	Hudson 1975
mallard ducklings	313 to 5000 in diet for 5 days	LD_{50} > 5000 ppm	Hudson 1975
bluegill sunfish [a]	300 mg/L for 96 hours	LC_{50} > 300 mg/L	Knapp and Terrell 1980
bluegill sunfish [a]	0.1 to 100 pm for 96 hours	LC_{50} > 100 mg/L	Rausina No Date
rainbow trout [a]	0.1 to 100 pm for 96 hours	LC_{50} > 100 mg/L NOEC = 10 mg/L	Rausina No Date
Daphnia [a, b]	0.01 to 0.22 mg/L for 96 hours	LC_{50} > 0.098 mg/L NOEC = 0.017 mg/L	LeBlanc et al. 1980
Eastern oysters [a]	1.25 to 20 mg/L for 96 hours	NOEC (new shell growth) = 20 mg/L	Ward 1981

[a] All values expressed a nominal rather than measured concentrations. See Section 4.1.3.3 for a discussion of the significance of nominal versus measured concentrations.
[b] Additional studies in *Daphnia* using water accommodated fractions of Disrupt II formulations have been conducted by Palmer and Krueger (2006a,b). The nominal concentrations reported in this study are not comparable to those reported above. See Section 4.3.3 for a more detailed discussion.

Table 4-2. Summary of QSAR Toxicity Estimates for Disparlure to Aquatic Species and Algorithms for Estimating the Toxicity of Mono-Epoxide Compounds to Aquatic Species Developed by Clements et Al. (1996).

Type of Estimate (Species)	Slope	Inter-cept	r^2 (n) [a]	Limiting Log_{10} Kow [b]	Estimated LC^{50} mg/L
Freshwater Acute					
Fish, 96h-LC_{50} (Fathead minnow)	0.382	-0.29	0.92 (4)	5	0.119
Fish, 16 day (Guppy)	0.246	-0.5	0.87 (9)	5	0.144
Invertebrate, 48h-LC_{50} (*Daphnia*)	-0.567	0.036	1.0 (2)	5	0.008

[a] Squared correlation coefficient and number of data points in analysis.
[b] These values are reported in the output of EPI Suite Version 3.12. Slightly different values are reported in Clements et al. (1996).

LIST OF APPENDICES

Appendix 1: Toxicity of disparlure to experimental mammals **(Unless otherwise specified, all concentrations are expressed as a.i.)**

Animal	Dose/Exposure	Response	Reference
ORAL - ACUTE			
rats, Sprague-Dawley 5 males, 5 females	single dose of 5000 mg a.i./kg (racemic preparation) by gavage. Animals observed for 15 days. No control group.	No mortalities. No microscopic abnormalities observed. Clinical signs of toxicity were piloerection, hunched posture and ungroomed appearance appearing on Day 1 of treatment. All signs were resolved by Day 4 of the observation period. **LD_{50} > 5000 mg a.i./kg**	Coleman 2000 MRID 45529801
rats, Sprague-Dawley albino	single dose of test material administered at several dose levels (10250, 15380, 23070, 34600 mg/kg) by gavage. Rats observed for 14 days following administration. No control group.	No mortality at any dose level. No gross pathological lesions at any dose level. At all dose levels, hypoactivity, ruffed fur, and diuresis were observed, **LD_{50} > 34600 mg a.i./kg**	Beroza et al. 1975 Hercon 1978 Kretchmar 1972 MRID 00128026
DERMAL			
rabbits, New Zealand	2025 mg/kg test material applied to shaved skin and occluded for 24 hours. Animals observed for 14 days for systemic toxicity	No mortalities. No gross pathologic lesions on necropsy. Local skin irritation after 24 hours (erythema and edema). 7 days after dosing, escharosis, desquamation, hemorrhaging and fissures. After 14 days, desquamation, fissures and pustules **LD_{50} > 2025 mg a.i./kg**	Beroza et al. 1975 Hercon 1978 Kretchman 1972 MRID 00128026

Appendix 1: Toxicity of disparlure to experimental mammals **(Unless otherwise specified, all concentrations are expressed as a.i.)**

Animal	Dose/Exposure	Response	Reference
rabbits, New Zealand	0.5 mL of undiluted test material (0.5 g) applied to shaved skin and occluded for 24 hours. Animals were observed for 72 hours	Primary dermal irritation study. Mild skin irritation (erythema and edema) was noted at 24 and 72 hours after application of test material	Beroza et al. 1975 Hercon 1978 Kretchman 1972 MRID 00128026
EYES			
6 young rabbits, New Zealand	0.1 mL undiluted sample (0.1 g) applied to conjunctival sac. Eye was not washed. Severity of ocular lesions was monitored at intervals of 24, 48, and 72 hours. Rabbits observed for 7 days.	3/6 rabbits had conjunctival redness at 24 hours. No effects observed in any rabbits at later times of the observation period	Beroza et al.1975 Hercon 1978 Kretchman 1972 MRID 00128026
INHALATION			
Albino rats (10)	Inhalation chamber study. Disparlure concentration 5.0 mg/L in air for 1 hour	No deaths were observed in this study. No assessment of sublethal toxicity was made **LC_{50}>5.0 mg a.i./L air**	Grapenthien 1972 MRID 00059821

Appendix 2: Toxicity of disparlure to birds **(unless otherwise specified, all doses and concentrations are expressed in terms of a.i.)**

Animal	Dose/Exposure	Response	Reference
bobwhite quail (5 months old)	Single oral doses of 398, 631, 1590, and 2510 mg/kg bw. Birds observed for 7 days after dosing	No mortalities at any dose level. No signs of toxicity associated with test material. At the highest dose, lethargy was observed in 3/10 birds on days 1-2 after dosing. Unclear if lethargy was related to test material. LD_{50} > 2510 mg/kg	Fink et al. 1980 MRID 00083102
bobwhite quail (12 day old chicks) mallard ducks (15 day old ducklings)	Dietary exposure to 313, 625, 1250,2500, 5000 ppm for 5 days. Birds observed for 3 days after end of dosing period	No mortalities in at any dose level for either species No signs of toxicity reported LC_{50} > 5000 ppm in diet for both quail and ducks	Hudson 1975 MRID 00105981 same data reported in MRID 00047225

Appendix 3: Toxicity of disparlure to aquatic species **(**unless otherwise specified, all concentrations are expressed in terms of a.i.)

Animal	Dose/Exposure	Response	Reference
FISH			
Rainbow trout Bluegills, 10 fish per concentration	0.1, 1.0, 10.0, 100.0 ppm (mg a.i./L) for 96 hours. Survival assessed at 1-6, 24, 48, 72, and 96 hours. Note: Very poor quality fiche. Dissolved oxygen was measured in the test water only when mortality was observed. The measurement itself cannot be read from the fiche.	No effect on dissolved oxygen. In bluegills, no affect on survivors at any concentration up to 96 hr exposure. **LC_{50}>100 ppm** In Rainbow trout, for all concentrations, no affect on survivors up to 48 hours. At the 100 ppm concentration, the number of survivors decreased to 8/10 after 72 hours of exposure. **LC_{50}>100 ppm**	Rausina No Date MRID 00059735
Bluegill sunfish, 30 fish in each group	Nominal concentration of 0 ppm (untreated control) and 300 ppm for 96 hours. No aeration during the study. No description of how the test water was prepared. No discussion of any observations concerning a surface film on the water.	No mortalities observed and no signs of altered behavior. Dissolved oxygen in test water and control water were comparable: Day 1 11.0 ppm (control) 10.4 ppm (test water) Day 4: 3.4 ppm (control) 3.4 ppm (test water) pH constant in test and control water (pH 6.4) of the duration of testing. **LC_{50}>300 ppm**	Knapp and Terrell 1980 MRID 00127869
AQUATIC INVERTEBRATES			
	Technical Grade Disparlure		
Eastern oysters (*Crassostrea virginica*)	96 hour exposure to concentrations ranging from 1.25 to 20 ppm 92% disparlure Acetone concentrations ranged up to 10%	No affect on new shell growth at any concentration **NOEC > 20 ppm**	Ward 1981 MRID 00074291

Appendix 3: Toxicity of disparlure to aquatic species **(**unless otherwise specified, all concentrations are expressed in terms of a.i.)

Animal	Dose/Exposure	Response	Reference
Daphnia magna, <24 hours old, 15 daphnids/concentration.	Disparlure TGAI 48-hour exposure to 0.010 - 0.22 mg/L [0.22, 0.13, 0.079, 0.048, 0.028, 0.017, and 0.01 mg/L nominal]. The concentration of disparlure in the test media was not measured. Static conditions in 500 mL test solution. Mortalities were recorded after 24 and 48 hours.	No mortalities or sublethal effects occurred at concentrations of 0.010 and 0.017 mg/L. Mortality rates at higher doses: 0.22 mg/L 15/15 0.13 mg/L 12/15 0.079 mg/L 2/15 0.048 mg/L 1/15 0.028 mg/L 1/15	LeBlanc et al. 1980 MRID 00127868

Additional notes on LeBlanc et al. 1980: Some organisms (number not specified) were trapped in the air-water interface at concentrations of 0.028 mg/L and higher. **EC_{50} = 0.098 (0.019-0.12) mg/L**.
NOEC = 0.017 mg/L

Standard Disrupt II Flakes (SF) – i.e., flakes previously used by FS

Daphnia magna, <24 hours old, 20 daphnids	Disrupt II, SF (**blank standard flakes, no disparlure**) 300 mg/L for 48 hours. 200 ml test solution volume	No mortality or immobility.	Palmer and Krueger 2006a
Daphnia magna, <24 hours old, 20 daphnids per concentration in 2 replicates with 10 organisms/replicate.	Disrupt II, SF 2003 (**standard flakes from 2003**, 17.9% disparlure) 0, 1, 3, 10, 30, 100, and 300 mg preparation/L. Preparations based on flakes mixed in water for 24 hours prior to the preparation of filtered test solutions (i.e., no flakes in the test solutions). Disparlure concentrations not monitored. The nominal formulation concentrations correspond to nominal concentrations of disparlure of: 0, 0.18, 0.54, 1.8, 5.4, 18, and 54 mg a.i./L.	No effects at any concentrations after 4 or 24 hours. At 48 hours, no effects at 1, 3, 30, and 100 mg formulation/L. At 10 mg/L, 1/20 organisms appeared lethargic. At 300 mg/L, 3/10 organisms in one replicate were trapped at the water surface but appeared normal after gentle submersion. 1/10 organisms did not appear normal (NOS) after being trapped on the water surface. EC_{50}: > 300 mg/L (53.7 mg a.i./L based on nominal concentrations)	Palmer and Krueger 2006a
Daphnia magna, <24 hours old, 20 daphnids per concentration in 2 replicates with 10 organisms/replicate.	Disrupt II, SF 2005 (**standard flakes from 2005**, 17.9% disparlure) 0, 1, 3, 10, 30, 100, and 300 mg preparation/L. Preparations based on flakes mixed in water for 24 hours prior to the preparation of filtered test solutions (i.e., no flakes in the test solutions). Disparlure concentrations not monitored. The nominal formulation concentrations correspond to nominal concentrations of disparlure of: 0, 0.18, 0.54, 1.8, 5.4, 18, and 54 mg a.i./L.	No effects at any concentrations after 4 hours. At 24 hours, 20 of 20 daphnids were either dead (n=3) or immobile (n=17) in the 300 mg/L group. No effects at lower concentrations. At 48-hours, no effects in the 1, 3, or 10 mg/L groups. At 30 mg/L, 9/20 organisms appeared to be lethargic. At 100 mg/L, 16/20 organisms were immobile. At 300 mg/L, 14/20 organisms were dead and the remaining 4 were immobile.	Palmer and Krueger 2006a

Additional Notes on Palmer and Krueger 2006a, (**standard flakes from 2005**): At 48 hours, no effects at 1, 3, 30, and 100 mg formulation/L. At 10 mg/L, 1/20 organisms appeared lethargic. At 300 mg/L, 3/10 organisms in one replicate were trapped at the water surface but appeared normal after gentle submersion. 1/10 organisms did not appear normal (NOS) after being trapped on the water surface.

24 hr LC_{50}: 173 (100-300 mg/L)
48 hr LC_{50}: 69 (30-100 mg/L)

Modified Disrupt II Flakes – i.e., flakes currently used by FS

Daphnia magna, <24 hours old, 20 daphnids	Disrupt II, MF (blank modified flakes, no disparlure) 300 mg/L for 48 hours. 200 ml test solution volume.	No mortality or immobility.	Palmer and Krueger 2006b
Daphnia magna, <24 hours old, 20 daphnids per concentration in 2 replicates with 10 organisms/replicate.	Disrupt II, MF 2003 (**modified flakes from 2003**, 17.9% disparlure) 0, 1, 3, 10, 30, 100, and 300 mg preparation/L. Preparations based on flakes mixed in water for 24 hours prior to the preparation of filtered test solutions (i.e., no flakes in the test solutions). Disparlure concentrations not monitored. The nominal formulation concentrations correspond to nominal disparlure concentrations of disparlure of: 0, 0.18, 0.54, 1.8, 5.4, 18, and 54 mg a.i./L.	At 4 hours, 1/20 daphnids in the 1 mg/L group trapped on the water surface but normal after gentle submersion. At 24 hours, no effects at any concentrations. At 48 hours, no effects at 3, 10, 30, and 100 mg formulation/L. At 1 mg/L and 300 mg/L, 2/20 daphnids in each group were trapped at the water surface but normal after gentle submersion. EC_{50}: > 300 mg/L	Palmer and Krueger 2006b
Daphnia magna, <24 hours old, 20 daphnids per concentration in 2 replicates with 10 organisms/replicate.	Disrupt II, MF 2005 (**modified flakes from 2005**, 17.9% disparlure) 0, 1, 3, 10, 30, 100, and 300 mg preparation/L. Preparations based on flakes mixed in water for 24 hours prior to the preparation of filtered test solutions (i.e., no flakes in the test solutions). Disparlure concentrations not monitored. The nominal formulation concentrations correspond to nominal concentrations of disparlure of: 0, 0.18, 0.54, 1.8, 5.4, 18, and 54 mg a.i./L.	At 4 hours, 17/20 daphnids in the 300 mg/L group trapped on the water surface but normal after gentle submersion. No effects at lower concentrations. At 24 hours: No effects in the 1, 3, 10, and 30 mg/L groups. At 100 mg/L, 14/20 dead and 6/20 trapped on the water surface. At 300 mg/L, 14/20 trapped on the water surface and lethargic after gentle submersion.	Palmer and Krueger 2006a

Modified Disrupt II Flakes – i.e., flakes currently used by FS

Daphnia magna, <24 hours old, 20 daphnids	Disrupt II, MF (blank modified flakes, no disparlure) 300 mg/L for 48 hours. 200 ml test solution volume.	No mortality or immobility.	Palmer and Krueger 2006b

Additional Notes, Palmer and Krueger 2006a. **Modified flakes, 2005**: At 48-hours, no effects in the 1, 3, or 10 mg/L groups. At 30 mg/L, 1/20 organisms appeared to be lethargic and 1/20 trapped on the water surface. At 100 mg/L, 20/20 organisms were dead. At 300 mg/L, 13/20 organisms were dead, 1/20 was lethargic, 2 were trapped on the water surface.

24 hr LC_{50}: > 30 mg/L

48 hr LC_{50}: 48 (30-100 mg/L)

Appendix 4: EPI Suite Output for Disparlure

Run conducted on June 28, 2006 by Patrick Durkin using EPI-Suite Version 3.12.

```
SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
CAS NUM: 029804-22-6
MOL FOR: C19 H38 O1
MOL WT : 282.51
----------------------------- EPI SUMMARY (v3.12) -------------------------
 Physical Property Inputs:
    Water Solubility (mg/L):   ------
    Vapor Pressure (mm Hg) :   ------
    Henry LC (atm-m3/mole) :   ------
    Log Kow (octanol-water):   ------
    Boiling Point (deg C)  :   ------
    Melting Point (deg C)  :   ------

KOWWIN Program (v1.67) Results:
===============================

                  Log Kow(version 1.67 estimate): 8.08

SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
MOL FOR: C19 H38 O1
MOL WT : 282.51
-------+-----+-------------------------------------------+---------+--------
 TYPE  | NUM |        LOGKOW FRAGMENT DESCRIPTION        |  COEFF  |  VALUE
-------+-----+-------------------------------------------+---------+--------
 Frag  |  3  |  -CH3    [aliphatic carbon]               | 0.5473  |  1.6419
 Frag  | 13  |  -CH2-   [aliphatic carbon]               | 0.4911  |  6.3843
 Frag  |  3  |  -CH     [aliphatic carbon]               | 0.3614  |  1.0842
 Frag  |  1  |  -O-     [oxygen, aliphatic attach]       |-1.2566  | -1.2566
 Const |     |  Equation Constant                        |         |  0.2290
-------+-----+-------------------------------------------+---------+--------
                                                        Log Kow   =   8.0828



MPBPWIN (v1.41) Program Results:
================================
Experimental Database Structure Match:  no data

SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
MOL FOR: C19 H38 O1
MOL WT : 282.51
----------------------- SUMMARY MPBPWIN v1.41 --------------------


Boiling Point:  328.27 deg C (Adapted Stein and Brown Method)

Melting Point:   56.00 deg C (Adapted Joback Method)
```

```
Melting Point:   78.02 deg C (Gold and Ogle Method)
Mean Melt Pt :   67.01 deg C (Joback; Gold,Ogle Methods)
  Selected MP:   67.01 deg C (Mean Value)

Vapor Pressure Estimations (25 deg C):
  (Using BP: 328.27 deg C (estimated))
  (Using MP: 67.01 deg C (estimated))
    VP:  0.00021 mm Hg (Antoine Method)
    VP:  0.000342 mm Hg (Modified Grain Method)
    VP:  0.000321 mm Hg (Mackay Method)
  Selected VP:  0.000342 mm Hg (Modified Grain Method)
```

TYPE	NUM	BOIL DESCRIPTION	COEFF	VALUE
Group	3	-CH3	21.98	65.94
Group	13	-CH2-	24.22	314.86
Group	1	>CH-	11.86	11.86
Group	2	>CH- (ring)	21.66	43.32
Group	1	-O- (ring)	32.98	32.98
*		Equation Constant		198.18
RESULT-uncorr		BOILING POINT in deg Kelvin		667.14
RESULT- corr		BOILING POINT in deg Kelvin		601.43
		BOILING POINT in deg C		328.27

TYPE	NUM	MELT DESCRIPTION	COEFF	VALUE
Group	3	-CH3	-5.10	-15.30
Group	13	-CH2-	11.27	146.51
Group	1	>CH-	12.64	12.64
Group	2	>CH- (ring)	19.88	39.76
Group	1	-O- (ring)	23.05	23.05
*		Equation Constant		122.50
RESULT		MELTING POINT in deg Kelvin		329.16
		MELTING POINT in deg C		56.00

```
Water Sol from Kow (WSKOW v1.41) Results:
=========================================

          Water Sol: 0.001939 mg/L

SMILES : O(C1CCCCCCCCC)C1CCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
MOL FOR: C19 H38 O1
MOL WT : 282.51
```

```
---------------------------------- WSKOW v1.41 Results ----------------------
--
Log Kow  (estimated)  :  8.08
Log Kow (experimental):  not available from database
Log Kow used by Water solubility estimates:  8.08

Equation Used to Make Water Sol estimate:
   Log S (mol/L) = 0.796 - 0.854 log Kow - 0.00728 MW + Correction
       (used when Melting Point NOT available)

      Correction(s):         Value
      --------------------   -----
       No Applicable Correction Factors
   Log Water Solubility  (in moles/L) :  -8.163
   Water Solubility at 25 deg C (mg/L):  0.001939


WATERNT Program (v1.01) Results:
================================

                  Water Sol (v1.01 est): 0.0027812 mg/L

SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
MOL FOR: C19 H38 O1
MOL WT : 282.51
-------+-----+-------------------------------------------+----------+-------
--
 TYPE  | NUM |    WATER SOLUBILITY FRAGMENT DESCRIPTION   |  COEFF   |  VALUE
-------+-----+-------------------------------------------+----------+-------
--
 Frag  |  3  |  -CH3    [aliphatic carbon]                |-0.3213   | -
0.9638
 Frag  | 13  |  -CH2-   [aliphatic carbon]                |-0.5370   | -
6.9812
 Frag  |  3  |  -CH     [aliphatic carbon]                |-0.5285   | -
1.5856
 Frag  |  1  |  -O-     [oxygen, aliphatic attach]        | 1.2746   |
1.2746
 Const |     |  Equation Constant                         |          |
0.2492
-------+-----+-------------------------------------------+----------+-------
--
                              Log Water Sol (moles/L) at 25 dec C  =   -
8.0068
                              Water Solubility (mg/L) at 25 dec C  =0.0027812
```

```
ECOSAR Program (v0.99h) Results:
================================
SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
CAS Num:
ChemID1:
ChemID2:
ChemID3:
MOL FOR: C19 H38 O1
MOL WT : 282.51
Log Kow: 8.08  (KowWin estimate)
Melt Pt:
Wat Sol: 0.0007897 mg/L  (calculated)
ECOSAR v0.99h Class(es) Found
-----------------------------
Epoxides
                                                                    Predicted
ECOSAR Class                  Organism            Duration  End Pt   mg/L
(ppm)
============================  ==================  ========  ======
==========
Neutral Organic SAR        : Fish                14-day    LC50      0.00192
*
(Baseline Toxicity)

Epoxides                   : Fish                96-hr     LC50        0.119
*
Epoxides                   : Fish                14-day    LC50        0.144
*
Epoxides                   : Daphnid             48-hr     LC50        0.008
*
 Note:  * = asterisk designates: Chemical may not be soluble
        enough to measure this predicted effect.
         Fish and daphnid acute toxicity log Kow cutoff: 5.0
         Green algal EC50 toxicity log Kow cutoff: 6.4
         Chronic toxicity log Kow cutoff: 8.0
         MW cutoff: 1000


HENRY (v3.10) Program Results:
==============================

       Bond Est :  1.49E-002 atm-m3/mole
       Group Est:  6.14E-002 atm-m3/mole

SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
MOL FOR: C19 H38 O1
MOL WT : 282.51
--------------------------- HENRYWIN v3.10 Results --------------------------
----------+-----------------------------------------+---------+----------
   CLASS  |     BOND CONTRIBUTION DESCRIPTION       | COMMENT |  VALUE
----------+-----------------------------------------+---------+----------
 HYDROGEN |  38  Hydrogen to Carbon (aliphatic) Bonds   |         | -4.5477
```

```
 FRAGMENT |  18  C-C                                          |         |  2.0935
 FRAGMENT |   2  C-O                                          |         |  2.1709
 FACTOR   |   *  Epoxide                                      |         |   .5000
----------+-----------------------------------------------+---------+----------
 RESULT   |    BOND ESTIMATION METHOD for LWAPC VALUE      |  TOTAL  |  0.217
----------+-----------------------------------------------+---------+----------
HENRYs LAW CONSTANT at 25 deg C = 1.49E-002 atm-m3/mole
                                = 6.07E-001 unitless

--------+-----------------------------------------------+------------+-------
-
        |        GROUP CONTRIBUTION DESCRIPTION         |   COMMENT  |  VALUE
--------+-----------------------------------------------+------------+-------
-
        |           3  CH3 (X)                          |            | -1.86
        |          13  CH2 (C)(C)                       |            | -1.95
        |           1  CH (C)(C)(C)                     |            |  0.24
        |           2  CH (C)(C)(O)                     |            |  0.24
        |           1  O (C)(C)                         |            |  2.93
--------+-----------------------------------------------+------------+-------
-
 RESULT |  GROUP ESTIMATION METHOD for LOG GAMMA VALUE  |    TOTAL   | -0.40
--------+-----------------------------------------------+------------+-------
-
HENRYs LAW CONSTANT at 25 deg C = 6.14E-002 atm-m3/mole
                                = 2.51E+000 unitless


Henrys LC [VP/WSol estimate using EPI values]:
    HLC:  6.556E-002 atm-m3/mole
    VP:   0.000342 mm Hg
    WS:   0.00194 mg/L


BIOWIN (v4.02) Program Results:
==============================
SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
MOL FOR: C19 H38 O1
MOL WT : 282.51
--------------------------- BIOWIN v4.02 Results ---------------------------

   Biowin1 (Linear Model Prediction)    :  Does Not Biodegrade Fast
   Biowin2 (Non-Linear Model Prediction):  Does Not Biodegrade Fast
   Biowin3 (Ultimate Biodegradation Timeframe):  Weeks
   Biowin4 (Primary  Biodegradation Timeframe):  Days-Weeks
   Biowin5 (MITI Linear Model Prediction)    :  Does Not Biodegrade Fast
   Biowin6 (MITI Non-Linear Model Prediction):  Does Not Biodegrade Fast
   Ready Biodegradability Prediction:  NO

------+-----+-----------------------------------------+---------+---------
 TYPE | NUM |       Biowin1 FRAGMENT DESCRIPTION      |  COEFF  |  VALUE
------+-----+-----------------------------------------+---------+---------
 Frag |  1  |  Linear C4 terminal chain  [CCC-CH3]     |  0.1084 |  0.1084
```

Frag	1	Aliphatic ether [C-O-C]	-0.3474	-0.3474
MolWt	*	Molecular Weight Parameter		-0.1345
Const	*	Equation Constant		0.7475
RESULT		Biowin1 (Linear Biodeg Probability)		0.3741

TYPE	NUM	Biowin2 FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	1	Linear C4 terminal chain [CCC-CH3]	1.8437	1.8437
Frag	1	Aliphatic ether [C-O-C]	-3.4294	-3.4294
MolWt	*	Molecular Weight Parameter		-4.0117
RESULT		Biowin2 (Non-Linear Biodeg Probability)		0.0699

A Probability Greater Than or Equal to 0.5 indicates --> Biodegrades Fast
A Probability Less Than 0.5 indicates --> Does NOT Biodegrade Fast

TYPE	NUM	Biowin3 FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	1	Linear C4 terminal chain [CCC-CH3]	0.2983	0.2983
Frag	1	Aliphatic ether [C-O-C]	-0.0087	-0.0087
MolWt	*	Molecular Weight Parameter		-0.6243
Const	*	Equation Constant		3.1992
RESULT		Biowin3 (Survey Model - Ultimate Biodeg)		2.8645

TYPE	NUM	Biowin4 FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	1	Linear C4 terminal chain [CCC-CH3]	0.2691	0.2691
Frag	1	Aliphatic ether [C-O-C]	-0.0097	-0.0097
MolWt	*	Molecular Weight Parameter		-0.4076
Const	*	Equation Constant		3.8477
RESULT		Biowin4 (Survey Model - Primary Biodeg)		3.6995

Result Classification: 5.00 -> hours 4.00 -> days 3.00 -> weeks
(Primary & Ultimate) 2.00 -> months 1.00 -> longer

TYPE	NUM	Biowin5 FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	1	Aliphatic ether [C-O-C]	0.0015	0.0015
Frag	3	Methyl [-CH3]	0.0004	0.0012
Frag	13	-CH2- [linear]	0.0494	0.6424
Frag	1	-CH- [linear]	-0.0507	-0.0507
Frag	2	-CH - [cyclic]	0.0124	0.0249
MolWt	*	Molecular Weight Parameter		-0.8405
Const	*	Equation Constant		0.7121

RESULT	Biowin5 (MITI Linear Biodeg Probability)		0.4910

TYPE	NUM	Biowin6 FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	1	Aliphatic ether [C-O-C]	-0.1071	-0.1071
Frag	3	Methyl [-CH3]	0.0194	0.0583
Frag	13	-CH2- [linear]	0.4295	5.5834
Frag	1	-CH- [linear]	-0.0998	-0.0998
Frag	2	-CH - [cyclic]	-0.1295	-0.2589
MolWt	*	Molecular Weight Parameter		-8.1558
RESULT	Biowin6 (MITI Non-Linear Biodeg Probability)			0.3883

A Probability Greater Than or Equal to 0.5 indicates --> Biodegrades Fast
A Probability Less Than 0.5 indicates --> Does NOT Biodegrade Fast

AOP Program (v1.91) Results:
==========

SMILES : O(C1CCCCCCCCC)C1CCCC(C)C
CHEM : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
MOL FOR: C19 H38 O1
MOL WT : 282.51

---------- SUMMARY (AOP v1.91): HYDROXYL RADICALS ----------

Hydrogen Abstraction = 21.7096 E-12 cm3/molecule-sec
Reaction with N, S and -OH = 0.0000 E-12 cm3/molecule-sec
Addition to Triple Bonds = 0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds = 0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings = 0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings = 0.0000 E-12 cm3/molecule-sec

OVERALL OH Rate Constant = 21.7096 E-12 cm3/molecule-sec
HALF-LIFE = 0.493 Days (12-hr day; 1.5E6 OH/cm3)
HALF-LIFE = 5.912 Hrs

---------- SUMMARY (AOP v1.91): OZONE REACTION ----------

****** NO OZONE REACTION ESTIMATION ******
(ONLY Olefins and Acetylenes are Estimated)

Experimental Database: NO Structure Matches

PCKOC Program (v1.66) Results:
==========

```
                  Koc (estimated): 3.44e+004


SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
MOL FOR: C19 H38 O1
MOL WT : 282.51
--------------------------- PCKOCWIN v1.66 Results -------------------------
-

         First Order Molecular Connectivity Index  ..... :  9.736
         Non-Corrected Log Koc  ........................... :  5.8004
         Fragment Correction(s):
                  1   Ether, aliphatic  (-C-O-C-)  .....  : -1.2643
         Corrected Log Koc  ............................... :  4.5361

                        Estimated Koc:  3.437e+004



HYDROWIN Program (v1.67) Results:
================================
SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
MOL FOR: C19 H38 O1
MOL WT : 282.51
--------------------------- HYDROWIN v1.67 Results -------------------------
-

NOTE: Fragment(s) on this compound are NOT available from the fragment
    library. Substitute(s) have been used!!!  Substitute R1, R2, R3,
    or R4 fragments are marked with double astericks "**".

                    O
                R1  / \  R3
EPOXIDE:          >C - C<
                R2       R4
          ** R1: n-Octyl-                ** R3: n-Butyl-
             R2: -H                         R4: -H
 Ka hydrolysis at (epoxy O) atom #  1:  4.271E-001  L/mol-sec

 Total Ka (acid-catalyzed) at 25 deg C :  4.271E-001  L/mol-sec
 Ka Half-Life at pH 7:     187.803  days

   The rate constant estimated for the EPOXIDE DOES NOT
   include the neutral hydrolysis rate constant!!
   For some epoxides, the neutral rate constant is the
   dominant hydrolysis rate at environmental pHs!
   If the neutral rate constant is important, the HYDRO
   estimated rate will under-estimate the actual rate!
```

```
BCF Program (v2.15) Results:
============================
SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
MOL FOR: C19 H38 O1
MOL WT : 282.51
---------------------------------- Bcfwin v2.15 -----------------------------
--
Log Kow  (estimated)  :  8.08
Log Kow (experimental):  not available from database
Log Kow used by BCF estimates:  8.08

Equation Used to Make BCF estimate:
   Log BCF = -1.37 log Kow + 14.4 + Correction

      Correction(s):                    Value
       Alkyl chains (8+ -CH2- groups)  -1.500

   Estimated Log BCF =  1.827  (BCF = 67.08)



                           Volatilization From Water
                           =========================

Chemical Name: Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-

Molecular Weight    :  282.51 g/mole
Water Solubility    :  -----
Vapor Pressure      :  -----
Henry's Law Constant:  0.0149 atm-m3/mole  (estimated by Bond SAR Method)

                             RIVER             LAKE
                           ---------         ---------
Water Depth     (meters):   1                 1
Wind Velocity    (m/sec):   5                 0.5
Current Velocity (m/sec):   1                 0.05

      HALF-LIFE (hours) :   1.781             160.4
      HALF-LIFE (days ) :   0.07422           6.682


STP Fugacity Model:  Predicted Fate in a Wastewater Treatment Facility
=======================================================================
   (using 10000 hr Bio P,A,S)
PROPERTIES OF: Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
-------------
Molecular weight (g/mol)                              282.51
Aqueous solubility (mg/l)                             0
Vapour pressure (Pa)                                  0
                (atm)                                 0
                (mm Hg)                               0
Henry 's law constant (Atm-m3/mol)                    0.0149
```

Air-water partition coefficient 0.609366
Octanol-water partition coefficient (Kow) 1.20226E+008
Log Kow 8.08
Biomass to water partition coefficient 2.40453E+007
Temperature [deg C] 25
Biodeg rate constants (h^-1),half life in biomass (h) and in 2000 mg/L MLSS (h):

-Primary tank	0.00	9999.79	10000.00
-Aeration tank	0.00	9999.79	10000.00
-Settling tank	0.00	9999.79	10000.00

STP Overall Chemical Mass Balance:

	g/h	mol/h	percent
Influent	1.00E+001	3.5E-002	100.00
Primary sludge	5.99E+000	2.1E-002	59.88
Waste sludge	3.33E+000	1.2E-002	33.28
Primary volatilization	2.72E-005	9.6E-008	0.00
Settling volatilization	6.01E-005	2.1E-007	0.00
Aeration off gas	9.17E-003	3.2E-005	0.09
Primary biodegradation	1.75E-002	6.2E-005	0.18
Settling biodegradation	4.25E-003	1.5E-005	0.04
Aeration biodegradation	5.60E-002	2.0E-004	0.56
Final water effluent	5.97E-001	2.1E-003	5.97
Total removal	9.40E+000	3.3E-002	94.03
Total biodegradation	7.77E-002	2.8E-004	0.78

STP Fugacity Model: Predicted Fate in a Wastewater Treatment Facility
==========
(using Biowin/EPA draft method)
PROPERTIES OF: Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-

Molecular weight (g/mol) 282.51
Aqueous solubility (mg/l) 0
Vapour pressure (Pa) 0
(atm) 0
(mm Hg) 0
Henry 's law constant (Atm-m3/mol) 0.0149
Air-water partition coefficient 0.609366
Octanol-water partition coefficient (Kow) 1.20226E+008
Log Kow 8.08
Biomass to water partition coefficient 2.40453E+007
Temperature [deg C] 25
Biodeg rate constants (h^-1),half life in biomass (h) and in 2000 mg/L MLSS (h):

-Primary tank	0.02	30.00	30.00
-Aeration tank	0.23	3.00	3.00
-Settling tank	0.23	3.00	3.00

STP Overall Chemical Mass Balance:

	g/h	mol/h	percent
Influent	1.00E+001	3.5E-002	100.00
Primary sludge	3.78E+000	1.3E-002	37.84
Waste sludge	3.83E-002	1.4E-004	0.38
Primary volatilization	1.72E-005	6.1E-008	0.00
Settling volatilization	6.92E-007	2.4E-009	0.00
Aeration off gas	1.14E-004	4.0E-007	0.00
Primary biodegradation	3.69E+000	1.3E-002	36.91
Settling biodegradation	1.63E-001	5.8E-004	1.63
Aeration biodegradation	2.32E+000	8.2E-003	23.16
Final water effluent	6.87E-003	2.4E-005	0.07
Total removal	9.99E+000	3.5E-002	99.93
Total biodegradation	6.17E+000	2.2E-002	61.70

(** Total removal recommended maximum is 99 percent)

Level III Fugacity Model (Full-Output):

Chem Name : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
Molecular Wt: 282.51
Henry's LC : 0.0149 atm-m3/mole (Henrywin program)
Vapor Press : 0.000342 mm Hg (Mpbpwin program)
Liquid VP : 0.00089 mm Hg (super-cooled)
Melting Pt : 67 deg C (Mpbpwin program)
Log Kow : 8.08 (Kowwin program)
Soil Koc : 4.93e+007 (calc by model)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.395	11.8	1000
Water	3.77	360	1000
Soil	28.1	720	1000
Sediment	67.8	3.24e+003	0

	Fugacity (atm)	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	1.26e-011	857	146	28.6	4.88
Water	4.55e-010	269	140	8.96	4.66
Soil	2.57e-012	1e+003	0	33.4	0
Sediment	2.8e-010	537	50.2	17.9	1.67

Persistence Time: 1.24e+003 hr
Reaction Time: 1.39e+003 hr
Advection Time: 1.1e+004 hr
Percent Reacted: 88.8
Percent Advected: 11.2

```
   Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
      Air:      11.82
      Water:    360
      Soil:     720
      Sediment: 3240
        Biowin estimate: 2.865  (weeks       )

   Advection Times (hr):
      Air:      100
      Water:    1000
      Sediment: 5e+004


-------------------------------------------------------------------------------
-
```

Appendix I
Diflubenzuron Risk Assessment

Figure I-1. The first power spraying apparatus was used in gypsy moth control operations before 1900.

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

Prepared for:

USDA, Forest Service

Forest Health Protection

GSA Contract No. **GS-10F-0082F**
USDA Forest Service BPA: **WO-01-3187-0150**
Requisition No.: **43-3187-1-0269**
Task No. **5**

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NOTE: Tables followed by figures are placed after Section 5 (References).

LIST OF WORKSHEETS

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AEL	adverse-effect level
a.i.	active ingredient
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
bw	body weight
CBI	confidential business information
CI	confidence interval
cm	centimeter
CNS	central nervous system
CPU	chlorophenylurea
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
EXAMS	Exposure Analysis Modeling System
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
GLEAMS	Groundwater Loading Effects of Agricultural Management Systems
ha	hectare
Hb	hemoglobin
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

ACRONYMS, ABBREVIATIONS, AND SYMBOLS (*continued*)

LOAEL	lowest-observed-adverse-effect level
LOC	level of concern
m	meter
M	male
MetHb	methemoglobinemia
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
mM	millimole
MOS	margin of safety
MRID	Master Record Identification Number
MSDS	material safety data sheet
MW	molecular weight
NCAP	Northwest Coalition for Alternatives to Pesticides
NCI	National Cancer Institute
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
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PRZM	Pesticide Root Zone Model
RBC	red blood cells
RED	re-registration eligibility decision
RfD	reference dose
SERA	Syracuse Environmental Research Associates
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
WP	wettable powder
µ	micron or micro-
4CA	4-chloroaniline

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

While the data base supporting the risk assessment of diflubenzuron is large and somewhat complex, the risk characterization is relatively simple. Diflubenzuron is an effective insecticide. Consequently, 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 of the gypsy moth. Some aquatic invertebrates may also be at risk; however, the risks appear to be less severe than risks to terrestrial insects. The risk characterization for aquatic invertebrates is highly dependant on site-specific conditions. In areas subject to minimal water contamination, the effects of diflubenzuron are expected to be marginally adverse or nonexistent. 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 humans and other groups of organisms—wildlife 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 of exposure (for example, a change in the availability of prey). There is no indication that 4-chloroaniline formed from the degradation of diflubenzuron will have an adverse effect on any species.

PROGRAM DESCRIPTION

Diflubenzuron is an insecticide that inhibits chitin deposition in arthropods and is effective either as a stomach or contact insecticide. Two formulations of diflubenzuron are labeled for control of the gypsy moth: Dimilin 4L and Dimilin 25W. Other formulations of diflubenzuron are available but these are registered for agricultural uses which account for about 94% of the total amount of diflubenzuron applied each year. Both ground and aerial applications of Dimilin 4L and Dimilin 25W are permitted. The current risk assessment concerns the range of labeled application rates—i.e., 0.0078-0.0624 lbs a.i./acre. Virtually all use of diflubenzuron in USDA programs occurs in suppression programs (about 99% of the treated acres) with only about 1% of the use in slow the spread programs. The use of diflubenzuron in eradication programs is less than 0.001% of the total use.

HUMAN HEALTH RISK ASSESSMENT

Hazard Identification **–** There is no information regarding effects in humans exposed to diflubenzuron; however, the toxicity of this compound is well characterized in experimental mammals. In mammals, the most sensitive effect involves damage to hemoglobin, a component of blood involved in the transport of oxygen. Diflubenzuron causes the formation of methemoglobin, a form of hemoglobin that is not able to transport oxygen. Methemoglobinemia, an excessive formation of methemoglobin, is the primary toxic effect of diflubenzuron regardless of the route or duration of exposure in every species of animal tested. Diflubenzuron 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 little indication that diflubenzuron causes other specific forms of toxicity. Diflubenzuron does not appear to be neurotoxic or immunotoxic, does not appear to affect endocrine function in laboratory mammals, and is not a carcinogen. In addition, diflubenzuron does not appear to cause birth defects or reproductive effects. 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. There are numerous studies regarding the subchronic and chronic toxicity of diflubenzuron in laboratory animals, and these studies indicate that methemoglobinemia is the most consistent and sensitive sign of toxicity. Diflubenzuron can be absorbed from the skin in sufficient amounts to cause hematological effects—that is, methemoglobinemia and sulfhemoglobinemia. Nonetheless, the dermal exposure concentrations that are necessary to cause these hematological effects are higher than the oral exposure doses that are necessary to cause the same effects.

Exposure Assessment – Exposure assessments are conducted for both diflubenzuron and 4-chloroaniline. For diflubenzuron, a standard set of exposure scenarios are presented for both workers and members of the general public. Concern for 4-chloroaniline arises because it is an environmental metabolite of diflubenzuron and is classified as a carcinogen. 4-Chloroaniline is not a concern in worker exposure assessments because 4-chloroaniline will not be present at the time that diflubenzuron is applied. Also, 4-chloroaniline is not a concern in some acute exposure scenarios for the general public such as direct spray during the application of diflubenzuron. Consequently, only a subset of the standard exposure scenarios—those associated with exposure to vegetation or water contaminated with diflubenzuron—are presented for 4-chloroaniline. These scenarios, however, include all standard chronic exposure scenarios, which are of greatest concern because of the potential carcinogenicity of 4-chloroaniline.

All exposure assessments are conducted at the maximum single application rate for diflubenzuron of 0.0625 lb/acre (equivalent to 70 g/ha). This is also the maximum application rate for a single season. Assuming that diflubenzuron is applied in a single application at the maximum rate 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.

For workers applying diflubenzuron, three types of application methods are considered: directed ground spray, broadcast ground spray, and aerial spray. Central estimates of exposure for workers are approximately 0.0009 mg/kg/day for aerial workers, 0.0008 mg/kg/day for backpack workers, and about 0.001 mg/kg/day for broadcast ground spray workers. Upper ranges of exposures are approximately 0.009 mg/kg/day for broadcast ground spray workers and 0.005 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 dose estimates that are either in the range of or substantially below the general exposure estimates for workers. The one exception involves wearing contaminated gloves for 1hour. The upper range of exposure for this scenario is about 0.4 mg/kg/day.

For the general public, estimates of acute exposure range from approximately 0.0000005 mg/kg, which is the lower range estimate for the consumption by a child of water from a stream contaminated by diflubenzuron, to 1.5 mg/kg, which represents the upper range for consumption of contaminated fish by subsistence populations—individuals who consume free-caught fish as a major proportion of their diet. Relatively high dose estimates are also associated with the consumption of contaminated water after an accidental spill (about 0.13 mg/kg at the upper range of exposure) and for the consumption of fish by members of the general public (0.3 mg/kg). Other acute exposures are lower by about an order of magnitude or more. For chronic or longer-term exposures, the modeled exposures are much lower than for acute exposures, ranging from approximately 0.00000002 mg/kg/day (2 in 10 millionths of a mg/kg/day), which is the lower range estimate for the consumption of contaminated water, to approximately 0.002 mg/kg/day, which is the upper range for consumption of contaminated fruit.

Estimates of exposure to 4-chloroaniline from contaminated vegetation are likely to be about 0.02 times less than 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.

Dose-Response Assessment – The dose-response assessment considers both diflubenzuron itself as well as 4-chloroaniline as an environmental metabolite of diflubenzuron. For systemic toxicity, the dose-response assessment involves the adoption or derivation of acute and chronic RfDs, doses that are considered to produce no adverse effects, even in sensitive individuals. RfDs are presented for both diflubenzuron and 4-chloroaniline. Cancer risk is considered quantitatively for 4-chloroaniline and is expressed as a dose associated with a risk of 1 in 1million. 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 derived a chronic RfD for diflubenzuron of 0.02 mg/kg/day. This chronic RfD is well documented and is used directly for all longer-term exposures to diflubenzuron. This value is based on a NOAEL in dogs and an uncertainty factor of 100. Because of the low acute toxicity of diflubenzuron, the U.S. EPA did not derive an acute RfD but identified an acute NOAEL of 10,000 mg/kg. While this NOAEL could be used to derive a surrogate acute RfD of 100 mg/kg, a more conservative approach is taken and a surrogate acute RfD of 11 mg/kg is derived based on a NOAEL of 1118 mg/kg from a study using a petroleum-based formulation of diflubenzuron. Since diflubenzuron is classified as a non-carcinogen by both U.S. EPA and WHO, there is no reason to conduct a quantitative cancer risk assessment for exposure to diflubenzuron.

The U.S. EPA derived a chronic RfD for 4-chloroaniline of 0.004 mg/kg/day, and this value is used in the current risk assessment 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 each for intraspecies extrapolation, sensitive subgroups, and the use of a LOAEL with an additional factor of 3 due to the lack of data reproductive toxicity data. As with diflubenzuron, the U.S. EPA did not derived an acute RfD for 4-chloroaniline. For this risk assessment 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—a factor of 10 for interspecies extrapolation, 10 for intraspecies extrapolation, and 3 for the lack of data on reproductive toxicity. 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 1.6×10^{-5} mg/kg/day that would be associated with a cancer risk of 1 in 1million.

Risk Characterization **–** The risk characterization for potential human health effects associated with the use of diflubenzuron in USDA programs to control the gypsy moth 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 basis for asserting 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, the assertion can be made that risk associated with exposure to diflubenzuron is essentially zero.

This 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.

ECOLOGICAL RISK ASSESSMENT

Hazard Identification **–** The toxicity of diflubenzuron is well characterized in most groups of animals, including mammals, birds, terrestrial invertebrates, fish, and aquatic invertebrates. In general, diflubenzuron is much more toxic to some invertebrates, specifically arthropods, than vertebrates or other groups of invertebrates. This differential toxicity appears to involve

fundamentally different mechanisms of action. Toxicity to sensitive invertebrate species is based on the inhibition of chitin synthesis. In the more tolerant vertebrate species, the mechanism of action appears to be a specific effect on the blood that inhibits oxygen transport.

The species most sensitive to diflubenzuron are arthropods, a large group of invertebrates, including insects, crustaceans, spiders, mites, and centipedes. Most of these organisms use chitin, a polymer (repeating series of connected chemical subunits) of a glucose-based molecule, as a major component of their exoskeleton—that is, outer body shell. Diflubenzuron is an effective insecticide because it inhibits the the formation of chitin. This effect disrupts the normal growth and development of insects and other arthropods. Both terrestrial and aquatic arthropods are affected but some substantial differences in sensitivity are apparent. In terrestrial organisms, the most sensitive species include lepidopteran and beetle larvae, grasshoppers and other herbivorous insects. More tolerant species include bees, flies, parasitic wasps, adult beetles, and sucking insects. In aquatic organisms, small crustaceans that consume algae and serve as a food source for fish (e.g., *Daphnia* species) appear to be the most sensitive to diflubenzuron, while larger insect species such as backswimmers and scavenger beetles are much less sensitive. A wide range of other aquatic invertebrates, other crustaceans ,and small to medium sized aquatic insect larvae, appear to have intermediate sensitivities. Not all invertebrates use chitin and these invertebrates are much less sensitive to diflubenzuron than the arthropods. For terrestrial invertebrates, relatively tolerant species include earthworms and snails. For aquatic species, tolerant species include ostracods and non-arthropods such as rotifers, bivalves (clams), aquatic worms, and snails.

The most sensitive effect in vertebrate species concerns damage to blood cells involved in the transport of oxygen. This effect was demonstrated in laboratory mammals used in toxicity studies (for example, rats and mice) as well as in domestic animals and livestock. Although the effect was not studied in wildlife mammals, birds, or fish, it is reasonable to assume that hemoglobin in all vertebrate species could be affected by exposure to diflubenzuron. Acute exposures to diflubenzuron are relatively non-toxic to mammals and birds. The U.S. EPA places diflubenzuron in low toxicity categories (III or IV) for mammals and considers diflubenzuron to be virtually non-toxic to birds in acute exposures and only slightly toxic to birds in subchronic exposures. This assessment is supported by a numerous field studies in which no direct toxic effects in mammals or birds is reported. Effects, if any, on terrestrial vertebrates from the application of diflubenzuron are likely to be secondary to changes in food availability—that is, reduced numbers of insects—or changes in habitat— for example, the loss of protective vegetation, relative to areas not treated with diflubenzuron. Aquatic vertebrates also appear to be relatively tolerant to diflubenzuron ,and this compound is classified by U.S. EPA as practically non-toxic to fish. This classification appears to be appropriate and is supported by several longer-term toxicity studies and field studies. Changes in fish populations are reported in some studies; however, the changes appear to be secondary to changes in food supply. Although the data on amphibians is much more limited than the data onfish, a similar pattern is apparent—that is, although there are no direct toxic effects from exposure, changes in food consumption patterns appear secondary to direct effects on invertebrate species.

Data on plants and microorganisms are more limited than the data on invertebrates or vertebrates. Nonetheless, there does not appear to be any basis for asserting that diflubenzuron will have a substantial effect on these organisms.

Exposure Assessment – As in the human health risk assessment, exposures are estimated for both diflubenzuron and 4-chloroaniline. A full set of exposure assessments are developed for diflubenzuron but only a subset of exposure assessments are developed for 4-chloroaniline. This approach is taken, again as in the human health risk assessment, because 4-chloroaniline is assessed as an environmental metabolite of diflubenzuron. Thus, immediately after application, the amount of 4-chloroaniline as an environmental metabolite will be negligible. Consequently, the direct spray scenarios as well as the consumption of insects and the consumption of small mammals after a direct spray are not included for 4-chloroaniline. Also as in the human health risk assessment, all standard chronic exposure scenarios are included for 4-chloroaniline.

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 diflubenzuron, the highest acute exposures for small terrestrial vertebrates will occur after a direct spray and could reach up to about 10 mg/kg at an application rate of 70 g/ha. Exposures anticipated from the consumption of contaminated vegetation by terrestrial animals range from central estimates of about 0.08 mg/kg for a small mammal to 2 mg/kg for a large bird with upper ranges of about 0.2 mg/kg for a small mammal and 5 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 range from approximately 0.001 to 0.005 mg/kg. Large birds feeding on contaminated vegetation at the application site could be exposed to much higher concentrations, ranging from about 0.08 to 0.7 mg/kg/day. The upper ranges of exposure from contaminated vegetation far exceed doses anticipated from the consumption of contaminated water, which range from about 0.0000001 to 0.00001 mg/kg/day for a small mammal.

Exposures of terrestrial organisms to 4-chloroaniline tend to be much lower than those for diflubenzuron. The highest acute exposure 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 is 0.0002 mg/kg/day, the dose associated with the consumption of contaminated vegetation by a large bird.

Exposures to aquatic organisms are based on essentially the same information used to assess the exposure to terrestrial species from contaminated water. At the maximum application rate of 70 g/ha, the upper range of the expected peak concentration of diflubenzuron in surface water is taken as 16 µg/L. The lower range of the concentration in ambient water is estimated at 0.01 µg/L. The central estimate of concentration of diflubenzuron in surface water is taken as 0.4 µg/L.

Dose-Response Assessment – Diflubenzuron 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 of 1118 mg/kg and a chronic NOAEL of 2 mg/kg/day. A similar approach is taken for 4-chloroaniline for which an acute NOAEL is 8 mg/kg is used based on a subchronic study and a chronic NOAEL is estimated at 1.25 mg/kg/day based on the chronic LOAEL of 12.5 mg/kg/day. For birds, the acute NOAEL for diflubenzuron is taken as 2500 mg/kg from an acute gavage study and the longer-term NOAEL is taken as 110 mg/kg/day from a reproduction study. No data are available regarding the toxicity of 4-chloroaniline in birds and the available toxicity values for mammals are used as a surrogate.

For terrestrial invertebrates two general types of data could be used to assess dose-response relationships: laboratory toxicity studies and field studies. Field studies are used in the current risk assessment 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 appear 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 22 g/ha. Somewhat high application rates—in the range of 30 to 35 g/ha—will adversely effect macrolepidoptera and some beneficial parasitic wasps. At the maximum application rate considered in this risk assessment— 70 g/ha—some 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 400 g/ha.

Invertebrates that do not synthesize chitin are also relatively tolerant to diflubenzuron. The NOEC for a species of earthworm (*Eisenia fetida*) is 780 mg/kg soil and is used to represent tolerant species of soil invertebrates. Very little information is available on the toxicity of 4-chloroaniline to terrestrial invertebrates. As with diflubenzuron, the earthworm appears to be relatively tolerant to 4-chloroaniline with a reported LC_{50} value of 540 mg/kg dry soil. The toxicity of both diflubenzuron and 4-chloroaniline to soil microorganisms is also relatively low.

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. For diflubenzuron, LC_{50} values of 25-500 mg/L are used to characterize risks for sensitive and tolerant species of fish, respectively. 4-Chloroaniline appears to be more toxic to fish and an LC_{50} of 2.4 mg/L is used to characterize risks of peak exposures, while an LC_{50} of 0.2 mg/L is used to characterize risks of longer-term exposures.

There is substantial variability in the response of different groups of aquatic invertebrates to diflubenzuron. Very small arthropods appear to be among the most sensitive species—with acute NOEC values ranging from 0.3 to about 1 ppb (µg/L) and chronic NOEC values ranging from 0.04 to 0.25 ppb. Based on acute NOEC values, larger arthropods, including crabs and

larger insects, appear to be more tolerant, with acute NOEC values ranging from 2 to 2000 ppb. For chronic effects, the differences between small and larger arthropods are less remarkable with stoneflies and mayflies (relatively large insects) having an NOEC value of 0.1 ppb, intermediate between *Daphnia* (0.04 ppb) and *Ceriodaphnia* (0.25 ppb). Molluscs (invertebrates including clams and snails) and worms (oligochaetes) appear to be much less sensitive to diflubenzuron.

The data on the toxicity of 4-chloroaniline to aquatic invertebrates is sparse. An acute NOEC of 0.013 mg/L is used to characterize acute risks associated with peak exposures in aquatic invertebrates, and an NOEC of 0.01 mg/L from a reproduction study is used to characterize longer-term risks to aquatic invertebrates.

Risk Characterization – While the data base supporting the risk assessment of diflubenzuron is large and somewhat complex, the risk characterization is relatively simple. Diflubenzuron is an effective insecticide. Consequently, 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 of exposure (for example, a change in the availability of prey). There is no indication that 4-chloroaniline formed from the degradation of diflubenzuron will have an adverse effect on any species

There is no indication that 4-chloroaniline formed from the degradation of diflubenzuron will have an adverse effects on any species.

1. INTRODUCTION

This document provides updated risk assessments for human health effects and ecological effects to support an assessment of the environmental consequences of using diflubenzuron for the control or eradication of the gypsy moth (*Lymantria dispar*) in USDA/Forest Service and USDA/APHIS programs. This risk assessment is an update to the human health and ecological risk assessments prepared for the 1995 Final Environmental Impact Statement (FEIS) for the Cooperative Gypsy Moth Management Program (USDA 1995).

In the preparation of this risk assessment, literature searches on diflubenzuron were conducted using PubMed, TOXLINE, AGRICOLA, as well as the U.S. EPA CBI files. There is a very large body of literature on the environmental fate and toxicology of diflubenzuron. In addition to the previous risk assessments (USDA 1995), the toxicology, environmental fate, and other aspects associated with the use of diflubenzuron are the subject of relatively comprehensive reviews of human health and ecological effects by the World Health Organization (WHO 1996; WHO 2001). Several other reviews of various topics involving diflubenzuron have been published in the open literature (e.g. Cunningham 1986; Eisler 1992; Fisher and Hall 1992; Wilson 1997) and in materials submitted to U.S. EPA (Cardona 1999; Hobson 2001; Lengen, 1999; Wilcox and Coffey 1978).

In addition, a large number of studies have been submitted to the U.S. EPA/OPP in support of the registration of diflubenzuron and most of these studies have been reviewed by U.S. EPA (U.S. EPA/OPP 1997a, 1997b, 2000) and the derivation of food tolerances (EPA/OPP 1999, 2002a, 2003). The U.S. EPA (1997a) re-registration eligibility decision (RED) document and other reviews by U.S. EPA include summaries of the product chemistry, mammalian toxicology, and ecotoxicology studies that were submitted by industry to the U.S. EPA. Full text copies of the studies most relevant to this risk assessment (n=118) were kindly provided by the U.S. EPA Office of Pesticide Programs. The CBI studies were reviewed, and synopses of the information that can be disclosed from these studies are included in this document.

While this document discusses the studies required to support the risk assessments, it makes no attempt to re-summarize all of the information cited in the existing reviews. This is a general approach in all Forest Service risk assessments. For diflubenzuron in particular, an attempt to re-summarize all of the available information would tend to obscure rather than clarify the key studies that should and do impact the risk assessment.

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

For the most part, the risk assessment methods used in this document are similar to those used in risk assessments previously conducted for the Forest Service as well as risk assessments conducted by other government agencies. Details regarding the specific methods used to prepare the human health risk assessment are provided in SERA (2001). 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 diflubenzuron and its commercial formulations, 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.

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

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

Variability reflects a knowledge or at least an explicit assumption about how things may change, while *uncertainty* reflects a lack of knowledge. For example, the focus of the human health dose-response assessment is an estimation of an 'acceptable' or 'no adverse effect' dose that will not be associated with adverse human health effects. For diflubenzuron and for most other chemicals, however, this estimation regarding human health must be based on data from experimental animal studies, which cover only a limited number of effects. Generally, judgment is the basis for the methods used to make the assessment. Although the judgments may reflect a consensus (i.e., be used by many groups in a reasonably consistent manner), the resulting

estimations of risk cannot be proven analytically. In other words, the estimates regarding risk involve uncertainty. The primary functional distinction between variability and uncertainty is that variability is expressed quantitatively, while uncertainty is generally expressed qualitatively.

In considering different forms of variability, almost no risk estimate presented in this document is given as a single number. Usually, risk is expressed as a central estimate and a range, which is sometimes very large. Because of the need to encompass many different types of exposure as well as the need to express the uncertainties in the assessment, this risk assessment involves numerous calculations. Some of the calculations are relatively simple are included in the body of the document. Some sets of the calculations, however, are cumbersome. For those calculations, worksheets are included with this risk assessment. The worksheets provide the detail for the estimates cited in the body of the document. Documentation for these worksheets is provided in a separate document (SERA 2003). A set of worksheets is provided for diflubenzuron (Supplement 1) as well as 4-chloroaniline (Supplement 2). As discussed in this risk assessment, 4-chloroaniline is a metabolite of diflubenzuron that is quantitatively considered in this risk assessment. Both sets of worksheets are provided with the hard-text copy of this risk assessment as well as with the electronic version of the risk assessment.

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). 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 diflubenzuron are defined in the text of this risk assessment.

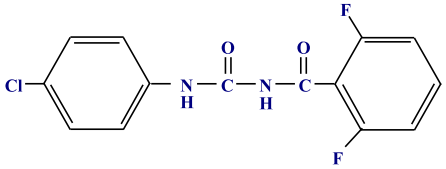
2. PROGRAM DESCRIPTION

2.1. Overview

Diflubenzuron is an insecticide that inhibits chitin deposition in arthropods and is effective either as a stomach or contact insecticide. Two formulations of diflubenzuron are labeled for control of the gypsy moth: Dimilin 4L and Dimilin 25W. Other formulations of diflubenzuron are available but these are registered for agricultural uses, which account for about 94% of the total amount of diflubenzuron applied each year. Both ground and aerial applications of Dimilin 4L and Dimilin 25W are permitted. For the current risk assessment, the range of labeled application rates – i.e., 0.0078 lb a.i./acre to 0.0624 lbs a.i./acre – are considered. Virtually all use of diflubenzuron in USDA programs occurs in suppression programs (about 99% of treated acres) with only about 1% of the use in slow the spread programs. The use of diflubenzuron in eradication programs is less than 0.001% of the total use.

2.2. Chemical Description and Commercial Formulations

Diflubenzuron is the common name for [1-(4-chlorophenyl) 3-(2,6-difluorobenzoyl)urea]:



Structurally, diflubenzuron consists of *p*-chloroanaline (the moiety on the left) linked to a 2,6-difluorobenzoic acid (the moiety on the right) by a ureido (carbon-nitrogen) bridge. Other synonyms for diflubenzuron as well as selected chemical and physical properties of diflubenzuron are summarized in Table 2-1. Additional information on the environmental fate and transport of diflubenzuron is summarized in the exposure assessments for the human health risk assessment (Section 3.2) and ecological risk assessment (Section 4.2).

Diflubenzuron is an insecticide that inhibits chitin deposition in arthropods and is effective either as a stomach or contact insecticide (Mabury and Crosby 1996). Chitin is a polymer (repeating series of connected chemical subunits) of a glucose-based molecule and comprises a substantial proportion of the exoskeleton (outer-shell) of arthropods. Consequently, the inhibition of chitin synthesis disrupts the growth and development (Baishya and Hazarika 1996; DeCleraq et al. 1995a,b; Griffith et al. 1996; Post and others 1974; Wright et al. 1996). Thus, diflubenzuron is not specific to the gypsy moth (Griffith et al. 1996; Horst and Walker 1995; Kadam et al. 1995) and is used to control a variety of pests on a variety of vegetation (Booth Riedl 1996; Boyle et al. 1996; McCasland et al. 1998). Because diflubenzuron can impact a number of invertebrate species, particularly aquatic species (e.g., Liber et al. 1996; O'Halloran et al. 1996), this compound is a restricted use pesticide that may only be applied by licenced applicators (C&P Press 2004).

Various formulations of diflubenzuron are labeled for forestry applications as well as other applications. All formulations of diflubenzuron are currently registered to Uniroyal Chemical (Table 2-2). Two formulations of diflubenzuron are labeled for control of the gypsy moth: Dimilin 4L and Dimilin 25W. As indicated in Table 2-2, an additional formulation, Micromite 25W, had been registered for gypsy moth but this formulation has been discontinued and the registration for this product has been canceled (U.S. EPA/OPP 2002b). Micromite 25WS and Micromite 25WGS are still available but these formulations are not used in USDA programs for the control of the gypsy moth.

Information on the impurities in and composition of these and other formulations of diflubenzuron have been submitted to U.S. EPA/OPP and this information (i.e., Drozdick 1998a,b,c,d,e; Van Kampen and Thus 1996; Vanstone 1998a,b,c; White 1998) has been reviewed as part of the current risk assessment. Specific information on inerts and contaminants in the diflubenzuron formulations is classified as CBI (confidential business Information) under Section 7(d) and Section (10) of FIFRA. This information cannot be specifically disclosed in this risk assessment. WHO (1996) has reported in the open literature that at least some processes in the synthesis diflubenzuron involve the reaction of 2,6-difluoro-benzamide with 4-chlorophenylisocyanate. Some inerts, however, must be disclosed on the material safety data sheet. Dimilin 4L contains petroleum oil [CAS No. 64742-46-7] and Dimilin 25W contains kaolin clay (C&P Press 2004). WHO (1996) indicated that kaolin is the only inert in some formulations of diflubenzuron. The potential risks associated with these inerts in the diflubenzuron formulations are discussed in Section 3.1.14.

2.3. Application Methods and Rates

Both ground and aerial applications of Dimilin 4L and Dimilin 25W are permitted (C&P Press 2004) and both methods are used in USDA programs. The most common methods for ground applications of diflubenzuron are 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/FS89b, p 2-9 to 2-10).

In some instances, directed foliar applications may be used. In selective foliar applications, backpack applicators are used and the insecticide is applied to 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, diflubenzuron formulations are applied under pressure through specially designed spray nozzles and booms. The nozzles are designed to minimize turbulence and maintain a large droplet size, both of which contribute to a reduction in spray drift. In aerial applications, approximately 40 to 100 acres may be treated per hour (USDA/FS89b, p 2-11). For Dimilin 25W, recommended droplet sizes are in the range of 150 to 200 microns (C&P Press 2004).

As indicated in Table 2-2, the application rate for Dimilin 4L ranges from 0.5 fluids ounces to 2 fluid ounces per acre. This corresponds to about 0.0039 to 0.0156 gallons [128 ounces per gallon] of Dimilin 4L per acre, which in turn corresponds to about 0.0156 to 0.0624 lbs diflubenzuron per acre [4 lbs diflubenzuron per gallon × 0.0039 to 0.0156] and 17 to 70 grams/ha. While multiple applications are permitted, the maximum single application rate is equal to the maximum annual application rate.

For Dimilin 25W, the range of labeled application rates is 0.5 ounces (avoirdupois) to 2 ounces per acre or 0.03125 to 0.125 pounds of Dimilin 25W per acre [i.e., 16 avoirdupois ounces per pound]. Since Dimilin 25W consists of 25% diflubenzuron, this range of application rates is equivalent to about 0.0078 to 0.03125 lb diflubenzuron per acre and 9 to 35 grams/ha. These rates for Dimilin 25W are about a factor of two below the corresponding rates for Dimilin 4L. The maximum application rate for Dimilin 25W in a single application is equivalent to the maximum annual application rate – i.e., multiple applications are allowed each year but the total amount applied in a single year cannot exceed 0.03125 lb a.i./acre [35 g/ha].

For the current risk assessment, the range of labeled application rates – i.e., 0.0078 lb a.i./acre to 0.0624 lbs a.i./acre – are considered. As calculated above, these rates are equivalent to 9 g/ha to 70 g/ha. All exposure assessments will be conducted at the maximum application rate. 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). These application rates are essentially the same as those used in the previous risk assessment (USDA 1995).

Recommended high volume ground sprays of Dimilin 4L and Dimilin 25W typically involve 100 to 400 gallons per acre but much concentrated solutions – i.e., 5 to 30 gallons per acre – are used in aerial applications. For the current risk assessment, the central value is taken as 30 gallons per acre and the range is taken as 5 to 400 gallons per acre. It should be noted that the selection of application rates and dilution volumes in this risk assessment is intended to simply reflect typical or central estimates as well as lower and upper ranges. In the assessment of specific program activities, the Forest Service will use program specific application rates in the worksheets that are included with this report to assess any potential risks for a proposed application.

The product label for Dimilin 25W specifically requires a 25 foot buffer for ground applications and a 150 foot buffer for aerial applications. These buffers indicate an area between the treated area and open bodies of water that may not be treated with diflubenzuron. The product label for Dimilin 4L does not specify a buffer but does indicate that the formulation cannot be applied to

water or *"...to areas where surface water is present"* (C&P Press 2004). In the aerial or ground applications, the USDA will use at least a 100 foot buffer and will extend the buffer up to 500 feet in some instances (Cook 2004).

2.4. Use Statistics

In order to minimize the ecological effects and human health effects of gypsy moth infestations, the USDA has adopted various intervention strategies that are roughly categorized as suppression, eradication, and slow the spread (USDA 1995). 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.

As indicated in Table 2-3, a total of 664,560 acres were treated with diflubenzuron formulations between 1995 and 2003, for an average annual treatment of about 73,840 acres per year. Virtually all (about 99%) of this use occurred in suppression programs with only about 1% of the use slow the spread programs. Very little diflubenzuron has been used in eradication programs – i.e., only 6 acres were treated in eradication programs accounting for <0.001% of the total acres treated for suppression, eradication, and slow the spread combined. Complete statistics for the amount of diflubenzuron applied in these applications has not been encountered. At the maximum labeled rate of 0.0624 lbs a.i./acre, the average annual treatment of about 73,840 acres per year would correspond to about 4608 pounds per year.

By comparison, the annual use of diflubenzuron on cotton for 1992 (the most recent year for which statistics are available) was 78,013 lbs (USGS 1998) or about a factor of 17 above the estimated average annul use by the Forest Service. The low use of the diflubenzuron by the USDA relative to agricultural applications – i.e., about 5.6% [4608 ÷ (78,013 + 4608) = 0.0558] – indicates that the use of diflubenzuron by the USDA will not contribute substantially to general levels of diflubenzuron in the environment. This 5.6% figure probably overestimates the use of diflubenzuron by the USDA relative to agricultural applications because USGS (1998) reports only use on cotton. Diflubenzuron is registered for application to a number of other agricultural crops. Nonetheless, localized release of diflubenzuron will occur and the consequences of this release is considered in the remainder of this risk assessment.

3. HUMAN RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION

3.1.1. Overview

No information is available on the effects of diflubenzuron on humans but the toxicity of this compound has been well characterized in experimental mammals. In mammals, the most sensitive effect involves damage to hemoglobin, a component of blood involved in the transport of oxygen. Diflubenzuron causes the formation of methemoglobin, a form of hemoglobin that is not able to transport oxygen. Methemoglobinemia, an excessive formation of methemoglobin, is the primary toxic effect of diflubenzuron by all routes of exposure and for all durations of exposure in all species of animals that have been tested. Diflubenzuron causes other effects on the blood but methemoglobinemia is the most sensitive effect – i.e., the effect that occurs at the lowest dose. While effects on the blood are well documented, there is little indication that diflubenzuron causes other specific forms of toxicity. Diflubenzuron does not appear to be neurotoxic or immunotoxic, does not appear to affect endocrine function in laboratory mammals, and is not a carcinogen. In addition, diflubenzuron does not appear to cause birth defects or reproductive effects. Diflubenzuron is relatively nontoxic by oral administration, with single-dose LD_{50} values reported as > 4640 mg/kg to >10,000 mg/kg. A large number of studies on the subchronic and chronic toxicity of diflubenzuron are available. As with acute toxicity, methemoglobinemia is the most consistent and sensitive sign of toxicity in laboratory mammals. Diflubenzuron can be absorbed from the skin in sufficient amounts to cause hematologic effects – e.g., methemoglobinemia and sulfhemoglobinemia. Nonetheless, these effects occur at higher doses after dermal administration than after oral administration.

3.1.2. Mechanisms of Action

Some specific mechanisms of action for diflubenzuron are well understood in both mammals and invertebrates. As discussed in Section 4.1, diflubenzuron inhibits chitin synthesis in invertebrates and this in turn disrupts normal growth and development and can lead to death. Mammals, including humans, do not produce chitin and this mechanism thus has no relevance to the human health risk assessment. Another mechanism of diflubenzuron involves damage to hemoglobin, a key component of blood, through the development of methemoglobin and sulfhemoglobin. This is highly relevant to the human health risk assessment and the formation of methemoglobin is the basis for the U.S. EPA RfD for diflubenzuron (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 (Hb) 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 to the ferric state (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. The most

common methemoglobin reductase is dependent on NADH, a molecule that is common in all living systems and is necessary for the proper function of many enzymes (Lo and Agar 1986). 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. As discussed further in Section 3.1.15 (Impurities and Metabolites), 4-chloroaniline, a metabolite of diflubenzuron, has also been shown to induce methemoglobinemia (WHO 2003).

Sulfhemoglobinemia is characterized by the presence of abnormal pigments, other than methemoglobin, in red cells and can be regarded as a form of nonspecific oxidative damage (Smith 1996) and, in some cases, the differential diagnosis of sulfhemoglobinemia and methemoglobinemia may be difficult (Demedts et al. 1997). As with methemoglobinemia, sulfhemoglobinemia can be induced by aromatic amines and hydroxyamines. Unlike methemoglobinemia, sulfhemoglobinemia is irreversible. Sulfhemoglobinemia is associated with the formation of Heinz bodies, dark-staining granules found in red blood cells. The formation of Heinz bodies can lead to red cell dysfunction and hemolysis (breakdown of the cell membrane). The damaged cells are in turn captured by the spleen, which can lead to spleen enlargement. In general, cats, mice, dogs, and humans are more susceptible to Heinz body formation compared with rabbits, monkeys, chickens, and guinea pigs (Smith 1996). Studies on the effects of diflubenzuron on methemoglobin, sulfhemoglobin, Heinz body formation, and the spleen are summarized in Appendix 1. These data are discussed in further detail in Section 3.3 (Dose-Response Assessment).

While diflubenzuron displays other types of toxicity, as discussed in the following subsections, the formation of methemoglobin and sulfhemoglobin are the only mechanisms of toxicity that have been clearly identified.

3.1.3. Kinetics and Metabolism

3.1.3.1. Oral Absorption **–** Diflubenzuron appears to be readily absorbed after oral administration but the extent of absorption is dose-dependant. Cameron et al. (1990) conducted a standard pharmacokinetic study on diflubenzuron in rats. Diflubenzuron was rapidly absorbed and excreted in both the urine and feces. Urine showed significant levels of 2,6-difluorobenzoic acid, 2,6-difluorophippuric acid, 2,6-difluorobenzaimide, 4-chlorophenyl urea, and 2'-hydroxydiflubenzuron. Fecal excretion contained mostly unchanged parent compound. 4-Chloroaniline was not detected in urine or bile (limit of detection = 7.5 ng/mL). As discussed further below, 4-chloroaniline is a metabolite of diflubenzuron in some species (Section 3.1.3.3) and is an environmental metabolite of diflubenzuron formed by biodegradation in soil. The oral absorption of diflubenzuron appears to be dependent on dose (e.g., Willems et al. 1980). At relatively low doses, in the range of 1 mg/kg/day, a substantial fraction of administered diflubenzuron (about 50%) is absorbed. At much higher doses, in the range of 1000 mg/kg/day, much less diflubenzuron is absorbed (about 5%) (WHO 1996, 2001). While studies on the basis for this dose-dependent absorption have not been located for diflubenzuron, this is a relatively common pattern in many compounds that are highly lipophilic – i.e., tend to concentrate in fat

tissue – and probably involves saturable transport by the lymphatic system (e.g., Rozman et al. 1979).

3.1.3.2. Dermal Absorption – No studies have been found on the dermal absorption of diflubenzuron in humans. Dermal absorption in rats has been studied by Andre (1996) and this study is summarized in Appendix 1. The dermal absorption of diflubenzuron appeared to be linear for doses of 0.005 or 0.05 mg/cm^2. This is unlike the pattern with oral absorption, as noted above, but the dermal doses are very low. In addition and unlike the case with oral absorption, there is no basis for asserting that dermal absorption is saturable. Andre (1996) does not provide a kinetic analysis of the absorption data. Andre (1996) does note that about 6% of the dose was bound to skin and that less than 1% of the dose was absorbed systemically over a 10 hour period. Taking 1% as an approximate measure of absorbed dose, the dermal absorption coefficient would be about 0.001 $hour^{-1}$ [$k = -\ln(1-0.01)/10\ hour = 0.001\ hour^{-1}$].

While several additional studies are available on the toxicity of diflubenzuron after dermal administration (Section 3.1.12.), these studies do not address the kinetics of dermal absorption. WHO (1996, 2001) summarizes an unpublished study conducted in the Netherlands indicating that 0.2% of a dermal dose of 150 mg/kg was absorbed by rabbits over a 6 hour exposure period. This corresponds to a dermal absorption rate of about 0.04 $hour^{-1}$ [$k = -\ln(1-0.002)/6\ hours = 0.000358\ hour^{-1}$], substantially less than the estimate in rats from the study by Andre (1996).

Estimates of first-order dermal absorption rates can also be made from structure activity relationships (SERA 2001). Based on these relationships, the estimated first-order dermal absorption rate for diflubenzuron is 0.0044 $hour^{-1}$ with a 95% confidence interval of 0.0019 $hour^{-1}$ to 0.01 $hour^{-1}$ (Worksheet A09). These estimate first-order dermal absorption rates are somewhat higher than those based on experimental measurements. The higher dermal absorption rates from Worksheet A09 are used in the current risk assessment. While this is a somewhat conservative or protective approach, it has little impact on the risk characterization (Section 3.4) because none of the exposures based on these conservative estimates approach a level on concern.

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 (SERA 2001). Using the method recommended by U.S. EPA/ORD (1992), the estimated dermal permeability coefficient for diflubenzuron is 0.012 cm/hour with a 95% confidence interval of 0.0066 to 0.021 cm/hour. The application of this method to diflubenzuron is given in Worksheet A10.

Note that the first-order and zero-order absorption coefficients are summarized in Worksheet 03 but are rounded to two significant places. Links to these values are used in all of the exposure worksheets involving dermal absorption.

3.1.3.3. Metabolism – Two types of metabolites are considered in this risk assessment: metabolites that are formed *in vivo* by an animal after diflubenzuron has been absorbed and metabolites that the formed in the environment through the degradation of diflubenzuron in environmental media – i.e., soil, air, and water. The *in vivo* metabolism of diflubenzuron has been reviewed by WHO (1996, 2001) and additional unpublished studies have been submitted to the U.S. EPA on the metabolism of diflubenzuron in rats (Cameron et al. 1990; Gay et al. 1999) as well as the environmental metabolism of diflubenzuron (Dzialo and Maynard 1999; Thus et al. 1991; Walstra and Joustra, 1990).

An overview of the *in vivo* and environmental metabolism of diflubenzuron is given in Figure 3-1. Two basic pathways exist for the metabolism of diflubenzuron. In the environment as well as in sheep, pigs, and chickens, the major route of metabolism involves cleavage of the ureido bridge with the formation of 2,6-difluorobenzoic acid and 4-chlorophenyl urea. The latter compound is then metabolized to 4-chloroaniline. As discussed further in Section 3.1.15, the formation of 4-chloroaniline is important to the human health risk assessment because this compound is classified as a carcinogen. The other pathway for the metabolism of diflubenzuron predominates in rats and cows and involves hydroxylation rather than cleavage of the ureido bridge. Hydroxylation of the aromatic rings involves the addition of a hydrogen-oxygen or hydroxy (OH) group to one of the rings. Hydroxylation increases the water solubility of aromatic compounds. Particularly when followed by conjugation with other water soluble compounds in the body, such as sugars or amino acids, hydroxylation greatly facilitates the elimination of the compound in the urine or bile. As detailed further by WHO (2001), the ureido bridge may also be cleaved in rats but 4-chloroaniline does not appear to be a major metabolite. No information has been located on the metabolism of diflubenzuron in humans.

3.1.4. Acute Oral Toxicity

No information has been found on the acute toxicity of diflubenzuron in humans. Information regarding the acute toxicity of diflubenzuron and diflubenzuron formulations in laboratory mammals is summarized in Appendix 1. These data indicate that diflubenzuron is relatively nontoxic by oral administration, with single dose LD_{50} values in mice and rats reported as > 4640 mg/kg to >10,000 mg/kg. In other words, less than half of the animals died at these doses. Many of the exposure scenarios considered in the current risk assessment for the use of diflubenzuron for the control of the gypsy moth do involve very short term acute exposures and the use of acute oral toxicity values is considered further in Section 3.3.3.

3.1.5. Subchronic and Chronic Toxicity

No information has been found on the subchronic or chronic toxicity of diflubenzuron in humans. A large number of studies using experimental mammals are available on the subchronic and chronic toxicity of diflubenzuron. Studies most relevant to the current risk assessment as summarized in Appendix 1 and additional information, including unpublished studies conducted in Europe, are summarized by WHO (1996, 2000).

As with acute toxicity, methemoglobinemia is the most consistent and sensitive sign of toxicity in laboratory mammals and has been observed in all mammalian species on which bioassays have been conducted: cats (Keet et al. 1982), dogs (Chesterman et al. 1974; Keet et al. 1982; Greenough et al. 1985), mice (Colley et al. 1981; Colley et al. 1984; Keet et al. 1984b), rats (Berberian and Enan 1989; Burdock et al. 1980; Burdock 1984; Keet et al. 1984a), and sheep (Keet et al. 1982).

For the current risk assessment, the most relevant longer-term toxicity study is the one-year oral toxicity study in which dogs were administered diflubenzuron in gelatin capsules at doses of 0, 2, 10, 50, or 250 mg/kg/bw (Greenough et al. 1985). As indicated in Appendix 1 and discussed further in Section 3.3.2, this is the study on which the U.S. EPA (1988; 1997a; 2000) has based the chronic RfD. In this study, no clinical signs of toxicity or pathology attributable to treatment were observed. The only adverse effects that were observed included dose-related increases in methemoglobin and sulfhemoglobin accompanied by an increase in spleen weight. As noted in the previous section, the increased spleen weight is probably secondary to the hematologic effects of diflubenzuron. This study is also important in that a clear duration-response relationship is apparent, with no significant changes in methemoglobin and sulfhemoglobin concentrations at four weeks after the start of dosing.

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.

Diflubenzuron, however, evidences few characteristics of a neurotoxicant even in terms of indirect effects. In an acute inhalation study involving a diflubenzuron formulation not used by the USDA (i.e, Dimilin 2L), excessive salivation and labored breathing were observed both during and after exposure (Hoffman 1997). While these can be signs of neurologic effects, they can be secondary to general irritation as well as other toxic effects. The only study on diflubenzuron that specifically assayed for neurotoxicity is the inhalation study by Newton (1999) in rats (details in Appendix 1). The neuro-behavioral battery included assays for autonomic effects, central nervous system effects (e.g., tremors and convulsions), general motor activity, movement and posture, reactivity to handling or sensory stimuli, grip strength, and observations for atypical behavior. Newton (1999) noted no treatment related effects of any endpoints assayed. The review of this study by WHO (2001) indicates that: "A reduction in 'grid count' was evident in the neuro-functional assessment of males and females exposed to 110 mg/m^3." Here, grid count refers to the number of grids that both front feet simultaneously touched during a fixed observations period. Based on the data reported in Newton (1999) for males (summary in Table 3, p. 44 and individual data in Appendix pp. 150-151 in Newton 1999)

and females (summary Table 3, p. 47 and individual data in Appendix pp. 168-169 in Newton 1999), a slight reduction in mean grid count is apparent for this response in study weeks 1, 2, and 3 but not in study week 4. There is, however, substantial scatter in the individual data in terms of the relationship of the response to concentration. The significance of the changes in grid count in the absence of any other sign of neurotoxicity is questionable.

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 diflubenzuron. The only studies specifically related to the effects of diflubenzuron on immune function are skin sensitization studies (Section 3.1.11). While the study by Blaszcak (1997e) indicates that diflubenzuron is not a skin sensitizer, this provides no information useful for directly assessing the potential for diflubenzuron to disrupt immune function.

Nonetheless, the toxicity of diflubenzuron 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 1). 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 at autopsy 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 diflubenzuron (Appendix 1).

3.1.8. Effects on Endocrine Function

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 diflubenzuron causes endocrine disruption in experimental mammals. Standard subchronic, chronic and reproductive toxicity studies provide no basis for asserting that any signs of overt toxicity are related to changes in endocrine function. As discussed further in Section 4, however, a few studies do indicate a potential endocrine effects in sheep (Section 4.1.2.1), birds (Section 4.1.2.2) and terrestrial insects (Section 4.1.2.3) but the strength of the association is limited.

3.1.9. Reproductive and Teratogenic Effects

Diflubenzuron has been tested for its ability to cause birth defects (i.e., teratogenicity) as well as its ability to cause reproductive and developmental impairment. Teratogenicity studies typically entail gavage administration to pregnant rats or rabbits on specific days of gestation. Two such studies (each of which is detailed in Appendix 1) were conducted on diflubenzuron: one in rats (Kavanagh 1988a) and one in rabbits (Kavanagh 1988a). As discussed by U.S. EPA/OPP (1997a), both of these were screening studies conducted at one very high dose, 1000 mg/kg bw. Since no signs of maternal or fetal toxicity were observed, no additional testing was required.

Another type of reproduction study involves exposing more than one generation of the test animal to the compound. One such study has been conducted on diflubenzuron (Brooker 1995). As detailed in Appendix 1, this study involved dietary exposures at concentrations of 0, 500, 5000, or 50,000 ppm over two generations in rats. No effects on reproductive performance were noted even though effects were seen on body weight (F_0 only) and increases were noted in methemoglobin and spleen weight – i.e., effects that may be attributable to diflubenzuron.

3.1.10 Carcinogenicity and Mutagenicity

There are no epidemiology studies or case reports that demonstrate or suggest that exposure to diflubenzuron leads to cancer in humans.

The carcinogenicity of diflubenzuron has been tested in rats and mice and these studies are detailed in Appendix 1. No carcinogenic effects were observed in rats exposed to diflubenzuron in a 2-year feeding study (Keet et al. 1984a). Neither treated nor control rats had cancers of any type, although pathological changes were observed in the spleen of both male and female rats. In mice, no carcinogenic effects or changes in spleen pathology were observed in males or females in a 2-year feeding study (Colley et al. 1984).

In addition to its lack of carcinogenic activity in *in vivo* bioassays, several bioassays of diflubenzuron for mutagenicity or other damage to DNA have failed to detect adverse effects. A lack of mutagenic activity has been reported in a dominant lethal study in mice (Arnold 1974), cell transformation assays using BALB/3T3 cells (Brusick and Weir 1977a), the induction of unscheduled DNA synthesis (Brusick and Weir 1977b), transplacental transformation assays using hamster cells (Quarles et al. 1980), and Ames assays using various strains of Salmonella typhimurium with and without metabolic activation (Brusick and Weir 1977c). Diflubenzuron did induce cell transformations in BALB/c 3T3 cells in the absence of metabolic activation; however, the effect was not observed with metabolic activation (Perocco and others 1993).

Diflubenzuron has been shown to inhibit the uptake of uridine, adenosine, and cytidine in cultured melanoma cells (Mayer et al. 1984) and inhibit the in vivo growth of melanomas in mice (Jenkins et al. 1986). Since the inhibition was enhanced by mixed function oxidase induction with 3-methylcholanthrene or beta-napthaflavone, aromatic hydroxylation was suggested as a requisite to tumor inhibition.

Both the U.S. EPA/OPP (1996a) and the WHO (1996, 2001) have concluded that diflubenzuron is not a carcinogen. This is detailed further in Section 3.3.2.3. However, the potential carcinogenicity of 4-chloroaniline, an environmental metabolite of diflubenzuron, is of concern and this is discussed further in Section 3.1.15 (Impurities and Metabolites) and in the dose-response assessment (Section 3.3.3.3).

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

As summarized in Appendix 1, diflubenzuron and formulations of diflubenzuron do not appear to be skin irritants (Blaszcak 1997d;) or sensitizers (Blaszcak 1997e). When instilled directly into the eye, however, diflubenzuron does cause slight to moderate conjunctival irritation (Blaszcak 1997c).

3.1.12. Systemic Toxic Effects from Dermal Exposure

As noted in Section 3.1.3.2, diflubenzuron can be absorbed from the skin and many of the exposure scenarios considered in this risk assessment involve dermal contact (Section 3.2). The available toxicity studies clearly indicate that diflubenzuron can be absorbed in sufficient

amounts to cause hematologic effects – e.g., methemoglobinemia and sulfhemoglobinemia (Goldenthal 1996). Nonetheless, these effects occur only at higher doses after dermal administration (i.e., 1000 mg/kg/day) than after oral administration (i.e., about 100 to 250 mg/kg/day). As with oral toxicity, severe signs of dermal toxicity are not observed even at doses that will induce methemoglobinemia and sulfhemoglobinemia (Blaszcak 1997b; Goldenthal 1996). This is an important relationship that impacts that characterization of risk, as detailed further in Section 3.4.

3.1.13. Inhalation Exposure

As with oral and dermal exposure, inhalation exposures appear to primarily effect the blood, causing increases in methemoglobin and sulfhemoglobin (Eyal 1999; Hoffman 1997; Berczy et al. 1975; Newton 1999). The threshold for these effects appears to be lower in nose only exposures – i.e., an NOEC of 30 mg/m^3 with an effect level of 100 mg/m^3 in the study by Eyal (1999) – compared to whole body exposures – i.e., an NOEC of 500 mg/m^3. It is unclear why this would be the case. In any event, as discussed further in Section 3.2, inhalation is not likely to be a significant route of exposure because of the low vapor pressure of diflubenzuron (Table 2-1) and ambient air will contain concentrations of diflubenzuron that are far below the NOEC values for nose-only exposure.

3.1.14. Inerts and Adjuvants

As noted in Section 2.2, Dimilin 4L contains petroleum oil [CAS No. 64742-46-7] and Dimilin 25W contains kaolin clay [CAS No. 1332-58-7] (C&P Press 2004). Kaolin clay is classified as a List 4a inert by the U.S. EPA (2004). This classification indicates that the product is considered as "Minimal risk inert ingredient". Petroleum oil with the CAS No. 64742-46-7 designation is classified as a List 2 inert which indicates a "Potentially Toxic Inert Ingredients/High Priority for Testing inerts". Details of these classifications may be found at: http://www.epa.gov/opprd001/inerts/lists.html. The toxicology of petroleum oil has been reviewed in some detail by ATSDR (2003). At sufficiently high doses, some petroleum oils can cause gastrointestinal, central nervous system (CNS), and renal effects. Petroleum oils however are highly variable and it is difficult to assess the potential contribution of the petroleum oil in Dimilin 4L to the overall toxicity of the formulation. No information on the toxicity of Dimilin 4L is included in the MSDS for this formulation (C&P Press 2004) or in the U.S. EPA RED (U.S. EPA/OPP 1997a) and no information on the toxicity of Dimilin 4L was encountered in the search of the U.S. EPA CBI files. The toxicity of Dimilin 2L (Blaszcak 1997a summarized in Appendix 1) appears to be comparable to that of Dimilin 25W (Koopman, 1977) as well as technical grade diflubenzuron (WHO 1996).

The identity of all inerts in both diflubenzuron formulations has been disclosed to the U.S. EPA (i.e., Drozdick 1998b,d; Vanstone 1998a,b,c) and this information has been reviewed as part of this risk assessment. This information, however, is protected under FIFRA (Section 10). Other than to state that no apparently hazardous materials have been identified, which is consistent with the MSDS for both Dimilin 4L and Dimilin 25W (C&P Press 2004), the information on the inerts in these formulations cannot be detailed.

3.1.15. Impurities and Metabolites

As with inerts, the impurities in formulations of diflubenzuron have been identified and disclosed to U.S. EPA (Drozdick 1998a,c,e; Van Kampen and Thus 1996; Vanstone 1998a,b,c; White 1998) and this information has been reviewed as part of this risk assessment. Again, this information is protected under FIFRA (Section 10) and cannot be disclosed in this risk assessment. Notwithstanding this limitation, the impurities that may be in diflubenzuron or formulations of diflubenzuron add relatively little uncertainty to this risk assessment. All toxicity studies summarized in Appendix 1 involved either technical grade diflubenzuron – i.e., diflubenzuron with any impurities – or the formulations which also contain the impurities. Thus, the available toxicity data should encompass the potential toxic effects of the impurities.

In terms of metabolites, the toxicity of most *in vivo* metabolites, as defined in Section 3.1.3.3, should also be encompassed by the available *in vivo* toxicity studies because these metabolites will be formed during the course of a standard *in vivo* toxicity study. This argument, however, does not hold for 4-chloroaniline for two reasons. First, as noted in Section 3.1.3.3, 4-chloroaniline does not appear to be metabolite in rodents, the species on which most toxicity studies have been conducted. Secondly, 4-chloroaniline is an environmental metabolite and is classified as a Group B2 carcinogen – i.e., indicating a probable human carcinogen following the classification of the U.S. EPA/OPP (1997a, 2000a) or a possible human carcinogen following the classification of the International Agency for Research on Cancer (IARC 1997). The carcinogenic activity of 4-chloroaniline has also been noted by WHO (2003). Consequently, potential exposures to 4-chloroaniline are quantitatively considered in the exposure assessment (Section 3.2), dose-response assessment (Section 3.3), and risk characterization (Section 3.4),

3.1.16. Toxicologic Interactions

There is no information on the interactions of diflubenzuron with other agents. Deleschuse et al. (1998) have investigated the cytotoxicity and induction of cytochromes P450 1A1/2 by insecticides in hepatic and epidermal cells. Diflubenzuron was one of the six pesticides studied and one of two that did not exert a cytotoxic effect in hepatocytes. In addition, de Sousa et al. (1997) noted a strong, dose-dependent, significant ($p<0.001$) induction of ethoxyresorufin O-deethylase (EROD) activity and or CYP1A1 mRNAs (5- to 7-fold greater than controls in human hepatocytes and approximately 7-fold greater than controls in rat hepatocytes). Any effect on hepatocytes and/or cytochrome P450 could impact how an organism would metabolize (either to toxicity or detoxify) a very large number of other compounds. The net effect of such interactions could be to enhance or inhibit toxicity and a more specific assessment would require data on specific combinations of other chemicals with diflubenzuron.

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview.

Exposure assessments are conducted for both diflubenzuron and 4-chloroaniline. For diflubenzuron, a standard set of exposure scenarios are presented for both workers and members of the general public. As discussed in the hazard identification, concern for 4-chloroaniline arises because it is an environmental metabolite of diflubenzuron and is classified as a carcinogen. Thus, 4-chloroaniline is not a concern in worker exposure assessments because 4-chloroaniline will not be present at the time that diflubenzuron is applied. Nor is 4-chloroaniline a concern in some acute exposure scenarios for the general public such as direct spray during the application of diflubenzuron. Consequently, only a subset of the standard exposure scenarios – those associated with contaminated vegetation and contaminated water – are presented for 4-chloroaniline but these do include all standard chronic exposure scenarios, which are of greatest concern because of the potential carcinogenicity of 4-chloroaniline.

All exposure assessments are based on the maximum single application rate for diflubenzuron of 0.0625 lb/acre. This is also the maximum application rate for a single season. Assuming that diflubenzuron is applied in a single application at the maximum rate 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.

For workers applying diflubenzuron, three types of application methods are considered: directed ground spray, broadcast ground spray, and aerial spray. Central estimates of exposure for workers are approximately 0.0009 mg/kg/day for aerial workers, 0.0008 mg/kg/day for backpack workers and about 0.001 mg/kg/day for broadcast ground spray workers. Upper range of exposures are approximately 0.009 mg/kg/day for broadcast ground spray workers and 0.005 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 where the upper range of exposure is about 0.4 mg/kg/day.

For the general public, the range of acute exposures is from approximately 0.0000005 mg/kg associated with the lower range for the consumption of contaminated water from a stream by a child to 1.5 mg/kg associated with the upper range for consumption of contaminated fish by subsistence populations – individuals who consume free-caught fish as a major proportion of their diet. Relatively high dose estimates are also associated with the consumption of contaminated water after an accidental spill (about 0.13 mg/kg at the upper range of exposure) and for the consumption of fish by members of the general public (0.3 mg/kg). Other acute exposures are lower by about an order of magnitude or greater. For chronic or longer term exposures, the modeled exposures are much lower than for acute exposures, ranging from approximately 0.00000002 mg/kg/day (2 in 10 millionths of a mg/kg/day) associated with the lower range for the consumption of contaminated water to approximately 0.002 mg/kg/day associated with the upper range for consumption of contaminated fruit.

Exposures to 4-chloroaniline from contaminated vegetation are likely to be below corresponding exposures to diflubenzuron by a factor of about 0.02. This follows from the expected rapid dissipation of 4-chloroaniline that is derived from diflubenzuron which has been deposited on vegetation. Estimated concentrations of 4-chloroaniline in water, however, are likely to equal or exceed anticipated concentrations of diflubenzuron under some circumstances. The peak exposures to 4-chloroaniline will occur at different times (later after the application of diflubenzuron) and under different conditions of precipitation than those of diflubenzuron. These differences are due to the relatively slow rate in the formation of 4-chloroaniline from diflubenzuron in soil.

3.2.2. Workers.

The Forest Service uses a standard set of exposure assessments in all risk assessment documents. All of the exposure assessments for workers as well as members of the general public are detailed in the worksheets on diflubenzuron 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 provide plain verbal descriptions of the worksheets and discuss diflubenzuron specific data that are used in the worksheets.

A summary of the exposure assessments for workers is presented in Worksheet E02 of the worksheets for diflubenzuron 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 the maximum single and maximum annual application rate of 0.0624 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 – 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.

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. These 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 diflubenzuron is 320 and the log K_{ow} is about 3.9. These values are within the range of the pesticides used in SERA (2001). As described 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 fast 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/FS 1989a,b,c). The number of hours worked per day is expressed as a range, the lower end of which is 6 hours 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.

Diflubenzuron can cause slight to moderate 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 1992a, 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 diflubenzuron is not available. Thus, the Kp for diflubenzuron is estimated using the algorithm from U.S. EPA (1992a), which is detailed in Worksheet A10.

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 diflubenzuron 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 more likely to be exposed to diflubenzuron. 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 diflubenzuron. These scenarios also assume that the child is completely covered with

diflubenzuron (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, as detailed in the Series B Worksheets.

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 diflubenzuron 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 for 24 hours. Other estimates used in this exposure scenario involve estimates of body weight, skin surface area, and first-order dermal absorption rates, as discussed in the previous section.

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 diflubenzuron 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 diflubenzuron in a small pond is estimated to range from about 0.014 mg/L to 1.1 mg/L with a central estimate of about 0.2 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 diflubenzuron 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 2004). If such a pond is directly sprayed with diflubenzuron at the nominal application rate of 0.0624 lb/acre, the peak concentration in the pond would be about 0.0035 mg/L (3.5 µg/L or 3.5 ppb) (Worksheet D10a). This concentration is a factor of about 300 below the peak concentration of 1.1 mg/L after the accidental spill. Because the USDA will not directly spray open bodies of water but will use buffers of 100 to 500 feet (Section 2.3), the concentration of 0.0035 mg/L from direct spray would be an accidental exposure. Using the 100 to 500 foot buffers, drift of diflubenzuron from aerial applications would result in water concentrations between about 7.7×10^{-06} mg/L (about 0.008 ppb or 8 ppt – parts per trillion) to about 6.8×10^{-05} mg/L (0.07 ppb or 70 ppt) (Worksheet 10a).

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 and it is assumed that the pesticide is applied along a 1038 foot length of the stream with a flow rate of 710,000 L/day. The length of 1038 feet is based on the length of a side of a square 10 ha treatment plot. At an application rate of 0.0624 lb/acre, accidental direct spray onto the surface of the stream would deposit about 4047 mg and this would result in a downstream concentration of about 0.0057 mg/L. Using a buffer of 100 feet, the drift would be a fraction of 0.0195 of the application rate (Worksheet B24) and the concentration in the stream would be about 0.00011 mg/L. Details of these and additional calculations for concentrations in stream water are given in Worksheet 10b.

3.2.3.4.3. Gleams Modeling – For compounds such as diflubenzuron, which may be applied to an entire 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 (2004).

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 (2004). 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 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 application rate of 0.0624 lb/acre (Section 2.3).

As indicated in Table 3-2, no stream contamination is estimated in very arid regions – i.e., annual rainfall of 10 inches of less. For regions with annual rainfall rates of 15 inches or more, the modeled peak concentrations in streams range from less than 0.01 µg/L (sandy soil) to about 15 µg/L (clay soil at an annual rainfall rate of 250 inches per year). While not detailed in Table 3-2, the losses from clay are associated almost exclusively with sediment loss (about 94%), with the remaining amount due to runoff. No water contamination due to percolation is modeled. This is consistent with a large body of literature on diflubenzuron indicating that downward movement in the soil horizon is extremely limited (e.g., Sundaram and Nott 1989; WHO 1996). Even in sandy soils, where very little water contamination is anticipated, percolation accounts for only about 3% 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 discussed further below, this is consistent with similar modeling conducted by Schocken et al. (2001) using PRZM/EXAMS. 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 0.004 µg/L (sandy soil) to about 3 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 B06b, direct spray of a standard pond could result in peak concentrations of about 3.5 µg/L, comparable to the peak levels modeled in ponds adjacent to fields with clay soil.

As discussed in Section 3.1.15, this risk assessment is also concerned with concentrations of 4-chloroaniline that could occur in water after the application of diflubenzuron. This process was also modeled using GLEAMS as described above for diflubenzuron. As illustrated in Figure 3-1, diflubenzuron does not degrade directly to 4-chloroaniline. It is first degraded to 4-chlorophenylurea which is in turn degraded to 4-chloroaniline. For the GLEAMS modeling, however, the degradation was modeled as a one-step process, disregarding the formation of 4-chlorophenylurea. This is a conservative approach in that the formation of 4-chlorophenylurea will attenuate the formation of 4-chloroaniline. As discussed further in the risk characterization (Section 3.4), this conservative approach has no impact on the risk assessment.

The chemical specific properties for 4-chloroaniline used in the GLEAMS modeling are given in Table 3-4 and the results for the stream and pond are summarized in Tables 3-5 and 3-6, respectively. The pattern seen with 4-chloroaniline is somewhat more complex than that seen with the parent compound. For example, the average and peak concentrations of 4-chloroaniline in streams is not directly related to rainfall rates (Table 3-5). The highest peak concentration, about 2 µg/L, occurs at a rainfall rate of 100 inches per year. At a rainfall rate of 250 inches per year, the modeled peak concentration is only about 0.36 µg/L. This pattern occurs because the formation of 4-chloroaniline is more rapid in soil than in water – i.e., great microbial activity in soil. Thus, at higher rainfall rates, diflubenzuron is washed rapidly from soil and lesser amounts of 4-chloroaniline are formed. A similar pattern with respect to rainfall rates is seen in the modeling results for the pond (Table 3-6).

The temporal exposures to 4-chloroaniline will also differ from those of diflubenzuron. This is illustrated in Figure 3-2 for concentrations of diflubenzuron and 4-chloroaniline in ponds at an annual rainfall rate of 150 inches. In clay and loam soils, diflubenzuron concentrations peak after the first rainfall and then steadily decline. Concentrations of 4-chloroaniline, however, peak after about 30 to 70 days. While diflubenzuron concentrations are much higher from clay than loam because of higher runoff from clay, the peak concentrations for 4-chloroaniline are similar for both clay (0.42 µg/L) and loam (0.35 µg/L), with the peak concentration in loam soil occurring somewhat later than that in clay soil. The greatest difference between diflubenzuron and 4-chloroaniline occurs in sand. As discussed above, virtually no diflubenzuron is expected to occur in ponds with very sandy soils. This is illustrated in Figure 3-2 for an annual rainfall of 150 inches, in which the concentration of diflubenzuron in water for sand is estimated at zero over the one-year model run. Nonetheless, 4-chloroaniline as a breakdown product from diflubenzuron will form and will rapidly leach through sand. Thus, for 4-chloroaniline, the peak concentrations in the pond with sandy soil, about 1.4 µg/L, are substantially higher than the peak concentrations associated with either clay or loam soils.

3.2.3.4.4. Other Modeling Efforts – A summary of the GLEAMS modeling discussed above as well as modeling of diflubenzuron conducted for other analyses is given in Table 3-7. While some of these modeling efforts involved assumptions 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. All of these modeling efforts used PRZM/EXAMS. As discussed in SERA (2004), PRZM (Pesticide Root Zone Model) is model used by U.S. EPA that is comparable to GLEAMS. PRZM is often linked with EXAMS (Exposure Analysis Modeling System) to estimate concentrations of pesticides in water (U.S. EPA/OPPTS 2004).

In the previous diflubenzuron risk assessment for the gypsy moth program (USDA 1995), maximum modeled concentrations at an application rate of 0.0624 lb/acre, identical to the rate used in the GLEAMS modeling, maximum concentrations in streams after direct spray were estimated at 16 ppb, very close to the estimate of 22 ppb made in the current risk assessment. Concentrations of diflubenzuron in streams associated with runoff were in the range of about 2

ppb to 13 ppb. These are very similar to the central and upper range of concentrations in streams based on the GLEAM modeling (2 ppb to 16 ppb). For open water, USDA (1995) estimated a maximum concentration of 1.22 ppb, which is only somewhat below the maximum of 3 ppb based on GLEAMS.

In the reregistration eligibility decision for diflubenzuron, U.S. EPA (1997a) modeled concentrations of diflubenzuron in surface water using Tier 2 computer models. These models are not otherwise specified in U.S. EPA (1997a). Typically, Tier 2 modeling by U.S. EPA involves PRZM/EXAMS. The U.S. EPA estimates much higher concentrations in water but this is largely due to differences in application rates. For example, at an application rate of 0.67 lb/acre, about a factor of 10 higher than the rate used with GLEAMS (0.0624 lb/acre), the U.S. EPA estimates a peak concentration of about 8.1 µg/L. Adjusting for the differences in application rate, the EPA estimate would be 0.8 µg/L [8.1 µg/L × 0.0624 lb/acre ÷ 0.67 lb/acre = 0.754 µg/L], similar to the estimates using GLEAMS with clay soil at rainfall rates of 100 to 150 inches. While the U.S. EPA (1997a) does not specify rainfall rates or soil types, Tier 2 modeling generally involves "worse case" assumptions which, in this case, would be based on high runoff soils (i.e., clay) and relatively high rainfall rates. The U.S. EPA (1997a) modeling for "Forestry" applications are specified as direct application. U.S. EPA (1997a) does not indicate the nature of the forestry application but direct spray of water does not correspond to applications for the control the gypsy moth. The concentrations modeled by U.S. EPA (1997a) of about 23 µg/L at an application rate of 0.07 lb/acre is consistent with the direct spray of a small stream modeled in this risk assessment (i.e., 22µg/L) but substantially higher than the direct spray of a pond (i.e., 3µg/L). In direct applications to shallow (1.3 to 1.7 m) ponds, Sundarum et al. (1991) monitored average day 1 concentrations in ponds of about 4 µg/L at an application rate of 70 g/ha (0.062 lb/acre), consistent with the peak concentrations in ponds discussed above (Section 3.2.3.4.3).

Harned and Relyea (1997) modeled diflubenzuron applications to a 10 ha plot after the application diflubenzuron at 350 g/ha, about a factor of 5 higher than the application rate used in the GLEAMS modeling. As with the EPA, Harned and Relyea (1997) used PRZM/EXAMS but combined these models with AgDrift. Harned and Relyea (1997) employed variable rainfall rates rather than fixed rates but the individual rainfall events varied from about 2.4 to 7.2 cm or about 1 to 2.8 inches. Based on their modeling, peak concentrations in the pond were estimated at about 1 µg/L. Correcting for the difference in application rates, their estimate of 1 µg/L would correspond to 0.2 µg/L in the GLEAMS modeling – i.e., higher by a factor of 5. As indicated in Table 3-3, concentrations estimated using GLEAMS at comparable daily rainfall events ranged from 0.2 to about 0.8 µg/L.

Schocken et al. (2001) also used AgDrift with PRZM/EXAMS to model diflubenzuron in streams and ponds beneath and adjacent to forests after an application of 0.125 lb/acre, about twice the application rate used in the GLEAMS modeling. Modeled estimates indicated that the initial concentration immediately after application should not exceed 0.255 µg/L in ponds and 0.938 µg/L in streams under the canopy. In adjacent areas, modeled estimates indicated that concentrations in ponds and streams should not exceed 0.260 µg/L and 0.856 µg/L, respectively.

The higher concentrations of diflubenzuron in streams compared to ponds is consistent with the GLEAMS modeling (Tables 3-2 and 3-3). The stream concentrations modeled by Schocken et al. (2001) of 1 µg/L are about a factor of 2 below the central estimates from GLEAMS – i.e., about 2 µg/L. This is probably due to the higher stream flow rate used by Schocken et al. (2001) – i.e., 58,320,000 L/day compared to 710,000 L/day used in the GLEAMS modeling. The peak concentrations in ponds modeled by Schocken et al. (2001), about 0.2 µg/L to 0.3 µg/L are very similar to the estimates from GLEAMS at rainfall rates of about 50 inches per year.

3.2.3.4.5. Monitoring Data – Several monitoring studies (Carr et al. 1991; Nigg and Stamper 1987; Van Den Berg 1986) are available that can be used to assess the plausibility of the modeling estimates summarized in Table 3-7. The common feature in each of these studies is that concentrations in pond and/or stream water are reported and these concentrations can be associated with a defined application rate. The study by Van Den Berg (1986) is probably the most directly relevant to this risk assessment. In this study, diflubenzuron was applied to a 10-acre mixed hardwood-conifer forested plot at an application rate of 0.0625 lb/acre. Initial concentrations of diflubenzuron in surface water (streams and stream pools) in treatment area ranged from 0.127-0.203 ppb and declined to 0.029-0.045 ppb after one day. These concentrations are in the range of concentrations modeled using GLEAMS for ponds (central range) and streams (lower range). Similar results are reported by Carr et al. (1991) who monitored concentrations in streams below 0.5 ppb after the application of diflubenzuron at rates of 13 g/ha or 26 g/ha. Adjusted for an application rate of 0.0624 lb/acre (70 g/ha), the concentration of 0.5 ppb would correspond to about 2.5 to 5 ppb, very close to the upper range of stream concentrations modeled using GLEAMS. The study by Nigg and Stamper (1987) involved a very high application rate, 560 g/ha (226 g/ac or 0.5 lb/acre) in a citrus grove. The maximum monitored concentration in an adjacent pond was 0.197 ppb. Adjusted to an application rate of 0.0624 lb/acre (70 g/ha), this corresponds to a concentration of about 0.02 ppb, in the lower range of pond concentrations modeled using GLEAMS.

This discussion of the monitoring data is not intended to imply a validation of the GLEAMS modeling or other modeling efforts. Model validation or calibration can only be done on a site-specific basis. Nonetheless, the monitoring data do suggest that estimates from GLEAMS as well as other comparable modeling efforts are at least plausible and may reasonably reflect the highly variable concentrations of diflubenzuron that may occur in surface water over a wide range of site-specific conditions.

3.2.3.4.6. Concentrations of Diflubenzuron in Water Used for Risk Assessment – A summary of the concentrations of diflubenzuron in water that are used for the current risk assessment is given in Table 3-8. The upper range of the expected peak concentration of diflubenzuron in surface water will be taken as 16 µg/L for an application rate of 0.0624 lb/acre. This is based on the upper range of concentrations estimated in streams from the GLEAMS modeling. This estimate is consistent with both the available monitoring data (Section 3.2.3.4.6) and other comparable modeling efforts (Section 3.2.3.4.5). This concentration also encompasses accidental direct sprays of both a small stream and small pond (Table 3-7). 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 diflubenzuron are likely to be essentially zero – i.e., applications made 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 concentration in ambient water will be set at 0.01 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 the concentration of diflubenzuron in surface water will be taken as 0.4 µg/L. This is the geometric mean of the range of 0.01 µg/L to 16 µg/L.

Longer term concentrations of diflubenzuron in surface water will be much lower than peak concentrations. At an application rate of 0.0624 lb/acre, the highest longer term concentration will be taken as 0.1 µg/L. This is near the maximum longer term concentration given by U.S. EPA (1997a) after adjusting for differences in application rate – i.e., 0.74 µg/L ÷ 6 applications at 0.06 lb/acre. This longer term maximum concentration is also near the upper range of the longer term concentrations modeled using GLEAMS – i.e., 0.06 µg/L in streams at an application rate of 0.0624 lb/acre. 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 0.001 µg/L, the lowest non-zero value modeled for diflubenzuron in ponds. This lower range is somewhat arbitrary but has no impact on the risk assessment. The central value for longer term concentrations of diflubenzuron in water will be taken as 0.02 µg/L. This is adapted from the longer term concentrations modeled by Harned and Relyea (1997) but adjusted for differences in the application rate – i.e., 0.1 µg/L × (70 g/ha ÷ 350 g/ha) = 0.02 µg/L. This value is similar to the central estimates of longer term concentrations in streams modeled using GLEAMS – i.e., 0.01 µg/L in Table 3-7 – but is near the upper range of concentrations that would be expected in ponds – i.e., 0.06 µg/L in Table 3-7.

3.2.3.4.7. Concentrations of 4-Chloroaniline in Water Used for Risk Assessment – A summary of the concentrations of 4-chloroaniline in water that are used for the current risk assessment is given in Table 3-9. The upper range of the expected peak concentration of 4-chloroaniline in surface water will be taken as 3 µg/L for an application rate of 0.0624 lb/acre. This is based on the upper range of concentrations estimated in streams near application sites with sandy soil over a range of annual rainfall rates from about 25 to 250 inches (Table 3-5). This concentration is higher than concentrations that might be expected in ponds by about a factor of 3 (Table 3-6). As with diflubenzuron, the lower range of concentrations of 4-chloroaniline in water will approach zero. For this risk assessment, the lower range is taken as 0.00003 µg/L, the lowest non-zero concentration modeled in ponds (i.e., Table 3-6, peak concentration for loam at an annual rainfall rate of 15 inches). The central estimate is taken as 0.5 µg/L. This is about the concentration modeled in stream with loam soil over a range of annual rainfall rates of 100 to 250 inches.

Longer term concentrations of 4-chloroaniline are taken as 0.05 µg/L with a range of 0.0002 µg/L to 0.2 µg/L at an application rate of 0.0624 lb/acre. The lower range is based on the lowest non-zero concentration modeled in ponds – i.e., loam soil at an annual rainfall rate of 15 inches.

The upper range is taken as the highest concentration modeled in ponds – i.e., sandy soil at annual rainfall rate of about 25 to 100 inches. The central estimate is based on the relatively narrow range of concentrations modeled in ponds with loam soil over rainfall rates of 50 to 250 inches per year – i.e., about 0.04 to 0.06 µg/L in Table 3-6. Much lower concentrations are likely to be seen in streams.

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).

Burgess (1989) assayed the bioconcentration of diflubenzuron in Bluegill sunfish, *Lepomis macrochirus*, over a 28 day exposure using C^{14}-labeled diflubenzuron. In this study, concentrations in water, whole fish, fillet (muscle), and viscera were measured at day 0.17 (4 hours), as well as on days 1, 3, 7, 14, 21, and 28. In fillet, the fish muscle ,the BCF was 120 after 1 day and 170 after 28 days with a peak of 200 after 7 days. In whole fish, the BCF was 260 after 1 day and 350 after 28 days with a peak of 360 after 7 days. Similar BCF values have been noted for diflubenzuron by Schaefer et al. (1979, 1980).

For the human health risk assessment of diflubenzuron, the BCF in fillet of 120 after 1 day will be used for acute exposures and the maximum BCF in fillet of 200 will be used for longer term exposures. This approach is taken under the assumption that humans will consume only the fish muscle. In the ecological risk assessment, however, the assumption will be made a predatory consumes the entire fish. Thus, for the ecological risk assessment, the whole body BCF values will be used, 260 for acute exposures and 360 for longer term exposures. These values are entered into Worksheet A02 for diflubenzuron and used in the subsequent worksheets involving exposures to contaminated fish.

Less detailed information is available on the bioconcentration of 4-chloroaniline. Because 4-chloroaniline is much more water soluble than diflubenzuron and has a much lower octanol-water partition coefficient, very little bioconcentration is expected in fillet or whole fish (WHO 2003). In a 14-day exposure of carp to two concentrations of 4-chloroaniline, Tsuda et al. (1993) noted essentially no bioconcentration – i.e., the concentrations in water were essentially identical to those in the fish. Thus, in Worksheet A02 for 4-chloroaniline, values of 1 are used for all BCF values – acute and chronic, whole fish and muscle.

For all scenarios involving the consumption of contaminated fish, concentrations of diflubenzuron or 4-chloroaniline in water are identical to the concentrations used in the

contaminated water scenarios (see Section 3.2.3.4). 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. Because of the available and well-documented information and substantial differences in the amount of caught fish consumed by the general public and native American subsistence populations, separate exposure estimates are made for these two groups (Worksheets D08a and D08b). The chronic exposure scenario is constructed in a similar way, as detailed in Worksheets D09a and D09b, except that estimates of concentrations in ambient water are based on the longer-term estimates summarized in Table 3-8 for diflubenzuron and Table 3-9 for 4-chloroaniline.

3.2.3.6. Oral Exposure from Contaminated Vegetation – Although Forest Service applications of diflubenzuron 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 two exposure scenarios developed for this exposure assessment include one scenario for acute exposure, as defined in Worksheet D03 and one scenario for longer-term exposure, as defined in Worksheet D04. In both scenarios, the concentration of diflubenzuron 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 B21. For the acute exposure scenario, the estimated residue level is taken as the product of the application rate and the residue rate (Worksheet D03).

For the longer-term exposure scenario (Worksheet D04), a duration of 90 days is used. The rate of decrease in the residues over time is taken from the vegetation half-time of 9.3 days (Table 2-1). Although the duration of exposure of 90 days is somewhat arbitrary, this duration is intended to represent the consumption of contaminated fruit that might be available over one season. Longer durations could be used for certain kinds of vegetation but would lower the estimated dose (i.e., would reduce the estimate of risk).

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 [$k=\ln(2)\div t_{50}$]. 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):

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

A somewhat different approach is required to assess exposures to 4-chloroaniline. Immediately after application, residues on vegetation will be comprised solely of diflubenzuron. As diflubenzuron degrades, 4-chloroaniline may be formed. Field studies, however, have indicated no residues of 4-chloroaniline on vegetation treated with diflubenzuron (Schroeder 1980). This may be due to the rapid atmospheric degradation of 4-chloroaniline in air – i.e., an estimated halftime of 3.9 hours or about 0.16 days. This is much less than the estimated vegetation halftime for diflubenzuron – i.e., 9.3 days (Sundaram 1986, 1996). Thus, the rate limiting step in the residues of 4-chloroaniline on vegetation will be the formation of 4-chloroaniline.

The approach for estimating concentrations of 4-chloroaniline on vegetation is conceptually similar to the approach taken with estimating concentrations in water. As a simplifying assumption, 4-chloroaniline generation will be estimated from the halftime of 9.3 days of diflubenzuron – i.e., direct breakdown from diflubenzuron to 4-chloroaniline. In addition, the dissipation of 4-chloroaniline from vegetation will be taken as the atmospheric halftime of 0.16 days, from WHO (2003). Under these conditions and at steady state, the ratio of 4-chloroaniline to diflubenzuron will be ratio of the these halftimes – i.e., 0.16 days ÷ 9.3 days = 0.017. In the scenario specific worksheets for 4-chloroaniline, all specific worksheets modeling exposure to contaminated vegetation are based on concentrations of diflubenzuron. The adjustment factor of 0.017 for 4-chloroaniline is incorporated into all worksheets involving exposure to contaminated vegetation (Worksheets D03, D04, F04a, F04b, F10, F11a, F11b, F12, F13a, F13b, F14a, and F14b).

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview

The dose-response assessment considers both diflubenzuron itself as well as 4-chloroaniline as an environmental metabolite of diflubenzuron. For systemic toxicity, the dose-response assessment involves the adoption or derivation of acute and chronic RfDs, doses that are considered to produce no adverse effects, even in sensitive individuals. RfDs are presented for both diflubenzuron and 4-chloroaniline. Cancer risk is considered quantitatively for 4-chloroaniline and is expressed as a dose associated with a risk of 1 in 1-million. 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 diflubenzuron of 0.02 mg/kg/day. This chronic RfD is well-documented and is used directly for all longer term exposures to diflubenzuron. This value is based on a NOAEL of 2 mg/kg/day in dogs and an uncertainty factor of 100 – a factor of 10 for interspecies differences and a factor of 10 for sensitive subgroups. Because of the low acute toxicity of diflubenzuron, the U.S. EPA has not derived an acute RfD but has identified an acute NOAEL of 10,000 mg/kg. While this NOAEL could be used to derive a surrogate acute RfD of 100 mg/kg, a more conservative approach is taken and a surrogate acute RfD of 11 mg/kg is derived based on a NOAEL of 1118 mg/kg from a study using a petroleum-based formulation of diflubenzuron. Diflubenzuron has been classified as a non-carcinogen by both U.S. EPA and WHO and no quantitative cancer risk assessment for exposures to diflubenzuron is conducted.

The U.S. EPA has derived a chronic RfD for 4-chloroaniline of 0.004 mg/kg/day and this value is used in the current risk assessment 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 data reproductive toxicity data. As with diflubenzuron, the U.S. EPA has not derived an acute RfD for 4-chloroaniline. For this risk assessment 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 – a factor of 10 for interspecies extrapolation, 10 for intraspecies extrapolation, and 3 for the lack of data on reproductive toxicity. 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 1.6×10^{-5} mg/kg/day that would be associated with a cancer risk of 1 in 1-million.

3.3.2. Diflubenzuron

3.3.2.1. Chronic RfD – The most recent RfD for diflubenzuron is 0.02 mg/kg/day. This value is given on the U.S. EPA's agency-wide list of approved RfDs (i.e., IRIS) (U.S. EPA 1990) and has been adopted by the U.S. EPA's Office of Pesticides (U.S. EPA/OPP 1997a,b, 2001a).

The chronic RfD is based on a study by Greenough et al. (1985) in which technical grade diflubenzuron was administered daily in gelatin capsules to dogs at doses of 0, 2, 10, 50, or 250 mg/kg/day, 7 days/week, for 52 consecutive weeks. At the lowest dose, 2 mg/kg/day, no effects were noted on methemoglobin formation or other standard endpoints. This study is detailed further in Appendix 1. The RfD was calculated by dividing the NOAEL of 2 mg/kg/day by 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 diflubenzuron.

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 diflubenzuron, the U.S. EPA (1997a) determined that the additional uncertainty factor is not required because of the information on the reproductive toxicity of diflubenzuron is adequate. As discussed in Section 3.1.9, diflubenzuron has been tested for and does not appear to cause birth defects or reproductive and developmental impairment.

For this risk assessment, the chronic RfD of 0.02 mg/kg/day is used to characterize risks for the general public as well as workers in longer term exposures. Because the RfD is intended to protect for lifetime exposures, it provides a conservative basis for comparing estimated exposure levels to an index of acceptable exposure.

3.3.2.2. Acute RfD – The U.S. EPA (1997a) did not specifically derive an acute RfD for diflubenzuron. In discussing the acute oral toxicity of diflubenzuron and referring specifically to the NOAEL of 10,000 mg diflubenzuron/kg bw from the single dose study in rats and mice by Koopman (1977) – i.e., a dose of 40,000 mg Dimilim/kg bw – the U.S. EPA/OPP (1996) concludes that:

> *One day single dose oral studies in rats and mice indicated only marginal effects on methemoglobin levels at a dose level of 10,000 mg/kg of a 25% wettable powder formulation. Sulfhemoglobin levels and Heinz bodies were not affected. Therefore, there is no acute dietary endpoint and a risk assessment for acute dietary exposure (1 day) is not necessary.* (U.S. EPA/OPP, 1996a, p. 16).

While this is a reasonable position, the current risk assessment is concerned with characterizing the risks of acute exposures as well as comparing the risks of acute exposures to diflubenzuron with risks associated with acute exposures other agents used to control the gypsy moth. A surrogate acute RfD of 100 mg/kg could be derived using the NOAEL of 10,000 mg/kg identified by U.S. EPA/OPP (1996a) and the uncertainty factor of 100 used by U.S. EPA/OPP (1996a) in deriving the chronic RfD (Section 3.3.2.1).

A more conservative approach, however, is taken for the current risk assessment. As noted in the hazard identification (Section 3.1.14), Dimilin 4L contains petroleum oil, a substance that is considered potentially toxic. While no acute toxicity studies have been encountered on Dimilin 4L, Blaszcak (1997a) has conducted a single dose gavage study in rats with Dimilin 2L, another petroleum based formulation of diflubenzuron. In this study, no signs of toxicity associated with treatment were noted at a dose of 5000 mg/kg as Dimilin 2L, equivalent to 1118 mg/kg as diflubenzuron. Thus, 1118 mg/kg rather than 10,000 mg/kg will be taken as the acute NOAEL. This value is used to calculate an acute RfD of 11 mg/kg by applying an uncertainty factor of 100, as in the chronic RfD, and rounding to the nearest integer.

3.3.2.3. Cancer Potency – The U.S. EPA/OPP (1996a) has determined that diflubenzuron itself does not pose a carcinogenic risk. Specifically, the U.S. EPA/OPP (1997a) has concluded that:

> *Based on the available evidence, which included adequate carcinogenicity studies in rats and mice and a battery of negative mutagenicity studies, diflubenzuron per se is classified as Group E (evidence of non-carcinogenicity for humans). –*
> (U.S. EPA 1997a, p. 18)

Thus, there is no basis for identifying carcinogenicity as and endpoint of concern and this effect is not treated quantitatively in the current risk assessment. This is consistent with the evaluation of the available data on carcinogenicity by WHO (1996, 2001).

3.3.3. 4-Chloroaniline

3.3.3.1. Chronic RfD – The chronic RfD for 4-chloroaniline is 0.004 mg/kg/day (U.S. EPA 1995). This RfD is based on a 2-year feeding study using rats in which the formation of non-neoplastic lesions of the splenic capsule was observed at 250 ppm in the diet (12.5 mg/kg/day) (NCI 1979). This dose is classified as a LOAEL and is divided by an uncertainty factor of 3,000 to derive the RfD. This uncertainty factor is intended to account for intra- and interspecies differences and the extrapolation from a LOAEL to a NOAEL. A value of ten is used for each of these three uncertainty factors is given – i.e., $10 \times 10 \times 10$. An additional factor of 3 was incorporated into the uncertainty factor because of the lack of supporting reproductive toxicity data. This data gap has also been noted by WHO (2003). Confidence in the principal study, the database for toxic effects, and the RfD itself is low (U.S. EPA 1995).

For this risk assessment, the chronic RfD derived by U.S. EPA (1995) is used for characterizing longer-term risks for the general public. As with the RfD for diflubenzuron, this provides a conservative basis for assessing the risks of longer term exposures, which are typically over periods far less than lifetime.

3.3.3.2. Acute RfD – As with diflubenzuron, the U.S. EPA has not proposed an acute RfD for 4-chloroaniline. As noted in Section 3.1, acute exposures to 4-chloroaniline are likely to be minimal immediately after the application of diflubenzuron – i.e., prior to the environmental

metabolism of diflubenzuron to 4-chloroaniline. Nonetheless, as detailed in Section 3.2.3.4 and illustrated in Figure 3-2, peak exposures to 4-chloroaniline in water may be higher than peak exposures to diflubenzuron in water, although the peak 4-chloroaniline exposures may occur weeks to months after the application of diflubenzuron. Consequently, this risk assessment will derive a surrogate acute RfD for 4-chloroaniline.

The toxicology of 4-chloroaniline has been reviewed in detail by WHO (2003) and the most relevant studies for the current risk assessment as summarized in Appendix 1. As a conservative approach, the surrogate acute RfD is based on the subchronic study by Scott and Eccleston (1967) in which rats were dosed daily with 4-chloroaniline at 0, 8.0, 20.0, or 50.0 mg/kg for 3 months. No hematologic or other adverse effects were observed at the lowest dose, 8 mg/kg/day. For the surrogate acute RfD, an uncertainty factor of 300 is used – a factor of 10 for interspecies extrapolation, 10 for intraspecies extrapolation, and 3 for the lack of data on reproductive toxicity. Thus, the surrogate acute RfD is taken as 0.03 mg/kg/day [8 mg/kg/day ÷ 300 = 0.02666 mg/kg/day which rounds to 0.03 mg/kg/day using one significant figure].

3.3.3.3. Cancer Potency – In the previous risk assessment for the use of diflubenzuron in gypsy moth programs (USDA 1995), a cancer potency factor of 0.013 $(mg/kg/day)^{-1}$ was used in the human health risk assessment. This was based on the NCI (1979) using the linearized multi-stage model. More recently, the U.S. EPA/OPP (1999, 2000a) has calculated a human cancer potency factor for 4-chloroaniline of 0.0638 $(mg/kg/day)^{-1}$, about a factor of 5 greater than the previous value used by USDA (1995).

In implementing the dietary risk assessment for the formation 4-chloroaniline from diflubenzuron, the U.S. EPA (2000a) has noted a potential cancer risk from 4-chlorophenylurea. As noted in Figure 3-1 and discussed in Section 3.1.3.3, 4-chlorophenylurea is structurally similar to 4-chloroaniline and is formed as an intermediate in the environmental breakdown of diflubenzuron to 4-chloroaniline. No specific information is available on the carcinogenicity of 4-chlorophenylurea. As a conservative approach in their dietary risk assessment of the degradation products of diflubenzuron, the U.S. EPA (2000a) elected to treat 4-chlorophenylurea as if it were a carcinogen with the same potency as 4-chloroaniline. This approach has been criticized by Cardona (1999, 2001) both because of the lack of information indicating that 4-chlorophenylurea is carcinogenic and because 4-chloroaniline does not appear to be an *in vivo* metabolite of 4-chlorophenylurea in rodents.

As detailed in Section 3.2.3.4.3 for drinking water and Section 3.2.3.6 for contaminated vegetation, the current risk assessment takes a somewhat different approach to the risks posed by 4-chlorophenylurea. There is no doubt that 4-chlorophenylurea is metabolized to 4-chloroaniline in the environment. Because the toxicity data on 4-chlorophenylurea are limited, the current risk assessment models the degradation of diflubenzuron to 4-chloroaniline as a one-step process, omitting the formation of 4-chlorophenylurea. While this is conceptually different from the equal potency assumption used by U.S. EPA (2000a), it is a conservative approach but avoids the

use of a surrogate potency parameter for a compound, 4-chlorophenylurea, for which there is no evidence of carcinogenicity.

For this risk assessment, the human cancer potency factor for 4-chloroaniline of $0.0638\ (mg/kg/day)^{-1}$ proposed by U.S. EPA/OPP (1999, 2000a) is used to assess cancer risks for all longer term exposure scenarios. This potency factor is not applied directly to any acute exposure assessments. Nonetheless, it is worth noting that all of the longer term estimates of exposure are based on average values that include short-term peak exposures. Thus, these higher but transient acute exposures are incorporated into the cancer risk assessment.

In the risk characterization worksheet for 4-chloroaniline (Worksheet E04 in Supplement 2), cancer risk is expressed as the ratio of exposure (dose in mg/kg/day) to a dose with a risk of 1 in 1-million. In a linear cancer model, such as that used by U.S. EPA, risk is assumed to be linearly related to dose:

$$\text{Risk} = \text{dose} \times \text{potency}$$

Thus, taking the potency factor of $0.0638\ (mg/kg/day)^{-1}$ and a risk level of 1 in 1-million (1×10^{-6}), the dose associated with a risk of 1 in 1-million can be calculated as:

$$\text{dose} = 1\times10^{-6} \div 0.0638\ (mg/kg/day)^{-1} = 0.000015673 \approx 1.6\times10^{-5}\ mg/kg/day$$

This dose is used in the Worksheet E04 for the risk characterization of cancer risks associated with exposure to 4-chloroaniline.

3.4. RISK CHARACTERIZATION

3.4.1. Overview

The risk characterization for potential human health effects associated with the use of diflubenzuron in USDA programs to control the gypsy moth 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 – i.e., assumptions that will tend to overestimate exposure – and because the dose-response assessment is based on similarly protective assumptions, there is no basis for asserting that this use of diflubenzuron poses a hazard to human health.

Notwithstanding the above assertion, it is worth noting that the greatest relative concern is with the contamination of water with 4-chloroaniline rather than with any exposures 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, the assertion can be made that risk associated with exposure to diflubenzuron is essentially zero.

This 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 of 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 1-million is 0.09, below the level of concern by a factor of 10.

3.4.2. Workers

A quantitative summary of the risk characterization for workers is presented in Worksheet E02 of the diflubenzuron worksheets (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 11 mg/kg is used (Section 3.3.3.2). 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 0.02 mg/kg/day is used (Section 3.3.3.1).

The qualitative risk characterization for workers is reasonably unequivocal. None of the acute or longer term hazard quotients exceed 1, the level of concern. In the normal application of diflubenzuron over the course of a season or even several years, the hazard quotients range from 0.04 to 0.07 – i.e., below the level of concern by factors of about 14 to 25. At the upper ranges of exposure for workers, the hazard quotients approach but do not exceed a level of concern – i.e., 0.2 to 0.5. Similarly, the upper range of hazard quotients for accidental/incidental exposures range from 0.0001 to 0.03, below the level of concern by factors of about 33 to 10,000. As noted in Section 3.2.2.2, the only accidental/incidental exposure that exceeds general exposures involves wearing contaminated gloves for 1 hour. While the hazard quotient of 0.03 is

substantially below a level of concern, the use of contaminated gloves appears to be the greatest source of concern in the handling of diflubenzuron.

Diflubenzuron can cause slight irritation to the eyes (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 diflubenzuron. This effect can be minimized or avoided by prudent industrial hygiene practices during the handling of the compound.

3.4.3. General Public

3.4.3.1. Diflubenzuron – A quantitative summary of the risk characterization for members of the general public is presented in Worksheet E04 of the diflubenzuron worksheets (Supplement 1). As with the risk characterization for workers, risk is expressed quantitatively as the hazard quotient using the surrogate acute RfD of 11 mg/kg (Section 3.3.3.2) and the chronic RfD of 0.02 mg/kg/day is used (Section 3.3.3.1).

Also as with workers, the qualitative risk characterization for members of the general public is unambiguous, with none of the acute or longer term hazard quotients exceeding 1 even at the upper ranges of plausible exposure. The highest hazard quotient is 0.1, the upper range of risk for the consumption of contaminated fish by subsistence populations. Nonetheless, this extreme 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 acute exposure, which involve somewhat less conservative assumptions, the acute hazard quotients range from 0.000003 to 0.02 – i.e., below the level of concern by factors of 50 to over 300,000.

3.4.3.2. 4-Chloroaniline – A quantitative summary of the risk characterization for members of the general public is presented in Worksheet E04 of the 4-chloroaniline worksheets (Supplement 2). Risk is expressed quantitatively as the hazard quotient using the surrogate acute RfD of 0.03 mg/kg (Section 3.3.3.2) and the chronic RfD of 0.004 mg/kg/day is used (Section 3.3.3.1).

In terms of both toxicity and carcinogenicity, the hazard quotients for members of the general public are comparable to but somewhat higher than the corresponding hazard quotients for diflubenzuron – a maximum hazard of 0.4 for 4-chloroaniline compared to a maximum hazard quotient of 0.1 for diflubenzuron.

The hazard quotient of 0.4 for 4-chloroaniline is associated with contamination of water, the hazard quotient for toxicity for the consumption of contaminated fish by subsistence populations and the hazard quotient for the dose associated with a cancer risk of 1 in 1-million for the longer term consumption of contaminated water. As detailed in Section 3.2.3.4 and illustrated in Figure 3-2, these risks are associated with the application of diflubenzuron to sandy soils in areas with annual rainfall rates of about 50 to 250 inches. In areas with predominantly clay or loam

soils, risks will be less by factors of about 3 to 10 (Table 3-6). Also, the relatively high hazard quotient of 0.4 is associated with standing bodies of water – i.e., ponds or lakes. Concentrations of 4-chloroaniline in streams even with sandy soil will be much less (Table 3-5).

Based on central estimates of exposure, acute hazard quotients range from 0.0004 to 0.01, below the level of concern by factors of 100 to 2500. Most chronic hazard quotients are in the range of 0.000002 to 0.0005, far below a level of concern. The only exception is the central estimate of the hazard quotient for the consumption of contaminated water based on a cancer risk of 1 in 1-million. This hazard quotient is 0.09, below the level of concern by a about a factor of 10. Nonetheless, the consumption of water that is contaminated with 4-chloroaniline as the greatest source of concern for members of the general public in the application of diflubenzuron to control the gypsy moth.

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 (Barretto et al. 1984). 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; Khakoo et al. 1993; Nilsson et al. 1990). A similar pattern is seen in many species of mammals (Lo and Agar 1986). Some infants with an intolerance to cow's milk or soy protein exhibit methemoglobinemia (Murray and Christie 1993; Wirth and Vogel 1988). These infants would be at increased risk if exposed to any materials contaminated with diflubenzuron or any compound that induces methemoglobinemia.

Individuals with poor diets may be at increased risk to some chemicals. Based on a study in rats (Hagler et al. 1981), iron deficiency leads to anemia but does not influence methemoglobin reductase activity. Thus, although individuals with poor nutritional status are generally a group for which there is particular concern, the available information does not support an increased concern for these individuals with respect to diflubenzuron exposure.

The RfDs used in the current risk assessment quantitatively consider sensitive subgroups. As noted in Section 3.3.2, the chronic RfD derived by U.S. EPA (1997a) incorporates a factor of 10 into overall uncertainty factor of 100 used for diflubenzuron to account for sensitive subgroups. Based on differences in methemoglobin reductase activity, a recovery mechanism for methemoglobinemia (Section 3.1.2), among different species, the factor of 10 for intraspecies variability appears adequate. The activity of this enzyme in humans appears to be about half of that in dogs (Calabrese 1991).

3.4.5. Connected Actions

The most sensitive effect for diflubenzuron, methemoglobinemia, is associated tebufenozide, 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. 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 diflubenzuron.

3.4.6. Cumulative Effects

This risk assessment is based on single applications at the maximum allowable rate, 70 g/ha. This is also the maximum rate that can be applied in a single season. This approach is used to estimate maximum daily exposure and daily absorbed dose. Because the dispersal rate for diflubenzuron in the environment is relatively fast, multiple applications at lower rates per application will result in risks that are less than those associated with a single application at the maximum approved rate. Given the narrow range of application rates compared with the variability and uncertainties in the exposure assessments, the risks of toxic effects associated with a single application at less than the maximum rate will be related directly to the application rate. Thus, an application at 35 g/ha will entail risks that are approximately one half of those expected at the maximum application rate.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview

The toxicity of diflubenzuron is well characterized in most groups of animals including mammals, birds, terrestrial invertebrates, fish and aquatic invertebrates. In general, diflubenzuron is much more toxic to some invertebrates, specifically arthropods, than vertebrates or other groups of invertebrates. This differential toxicity appears to involve fundamentally different mechanisms of action. Toxicity to sensitive invertebrate species is based on the inhibition of chitin synthesis. In the more tolerant vertebrate species, the mechanism of action appears to be a specific effect on the blood that inhibits oxygen transport.

The species most sensitive to diflubenzuron are arthropods, a large group of invertebrates including insects, crustaceans, spiders, mites, and centipedes. Most of these organisms use chitin, a polymer (repeating series of connected chemical subunits) of a glucose-based molecule, as a major component of their exoskeleton – i.e., outer body shell. Diflubenzuron is an effective insecticide because it inhibits the the formation of chitin. This effect disrupts the normal growth and development of insects and other arthropods. Both terrestrial and aquatic arthropods are affected but some substantial differences in sensitivity are apparent. In terrestrial organisms, the most sensitive species include lepidopteran and beetle larvae, grasshoppers and other herbivorous insects. More tolerant species include bees, flies, parasitic wasps, adult beetles, and sucking insects. In aquatic organisms, small crustaceans that consume algae and serve as a food source for fish (e.g., *Daphnia* species) appear to be the most sensitive to diflubenzuron while larger insect species such as backswimmers and scavenger beetles are much less sensitive. A wide range of other aquatic invertebrates, other crustaceans and small to medium sized aquatic insect larvae, appear to have intermediate sensitivities. Not all invertebrates utilize chitin and these invertebrates are much less sensitive to diflubenzuron than the arthropods. For terrestrial invertebrates, relatively tolerant species include earthworms and snails. For aquatic species, tolerant species include ostracods (an arthropod) and non-arthropods such as rotifers, bivalves (clams), aquatic worms, and snails.

As detailed in the human health risk assessment, the most sensitive effect in vertebrate species appears to involve damage to blood cells involved in the transport of oxygen. This effect has been demonstrated in mammals that are often employed in toxicity studies (e.g., rats and mice) as well as domestic animals and livestock. The effect has not been demonstrated in wildlife mammals, birds, or fish but it seems reasonable to assume that hemoglobin in all vertebrate species could be affected by exposure to diflubenzuron. Acute exposures to diflubenzuron are relatively non-toxic to mammals and birds. The U.S. EPA places diflubenzuron in low toxicity categories (III or IV) for mammals and considers diflubenzuron to be virtually non-toxic to birds in acute exposures and only slightly toxic to birds in subchronic exposures. This assessment is supported by a large number of field studies in which no direct toxic effects in mammals or birds have been reported. Effects, if any, on terrestrial vertebrates from the application of diflubenzuron are likely to be secondary to changes in food availability (i.e., reduced numbers of

insects) or changes in habitat (i.e., the protection of vegetation relative to untreated areas). Aquatic vertebrates also appear to be relatively tolerant to diflubenzuron and this compound is classified by U.S. EPA as practically non-toxic to fish. This classification appears to be appropriate and is supported by a relatively large number of longer term toxicity studies as well as field studies. Changes in fish populations have been noted in some studies but the changes appear to be secondary to changes in food supply. Although the data on amphibians are much more limited than the data in fish, a similar pattern is apparent – i.e., no direct toxic effects but changes in food consumption patterns secondary to effects on invertebrate species.

Data on plants and microorganisms are more limited than the data on invertebrates or vertebrates. Nonetheless, there does not appear to any basis for asserting that diflubenzuron will have a substantial effect on these organisms.

4.1.2. Toxicity to Terrestrial Organisms

4.1.2.1. Mammals – As summarized in Appendix 1 and discussed in the human health risk assessment (Section 3.1), there are a large number of toxicity studies on diflubenzuron in experimental mammals and these studies are relevant to the risk assessment for terrestrial mammals. Potential hazard to all wildlife mammals, however, may not be encompassed by the available data on experimental mammals – i.e., rats, mice, and dogs. As discussed in Section 3.1.3.1 and illustrated in Figure 3-1, some mammals such as sheep and pigs will metabolize diflubenzuron differently from rats. Specifically, metabolism in sheep, pigs, and perhaps other mammalian species, will result in cleavage of the ureido bridge with the formation of metabolites that are different from those seen in rats. There is little indication, however, that this difference in metabolism will lead to marked differences in toxicity. As summarized in Appendix 1, substantial differences in sensitivity among different species of mammals are not apparent. One possibly noteworthy difference, however, is a reduction in thyroid weight in sheep (Ross et al. 1977). As discussed in Section 3.1.8, the thyroid is an important organ in endocrine function. This effect, however, occurred in the absence of any signs of toxicity or changes in growth and may have been incidental.

The available field studies do not indicate any substantial impacts on mammalian wildlife from applications of diflubenzuron (Appendix 3a). As summarized in USDA (1995), applications of 60 to 280 g a.i./ha (0.85 to 4 oz a.i./ac) had no detectable adverse effects on the abundance of or reproduction in voles, field mice, and shrews (O'Connor and Moore 1975; Henderson et al. 1977). Small mammals increased in abundance on a plot receiving 280 g a.i./ha compared with a control plot (Henderson et al. 1977). The adverse effect that diflubenzuron might have on bot flies, a parasite of small as well as large mammals, was suggested as a possible explanation.

A more recent published field study by Seidel and Whitmore (1995) reports no effects on body measurements, weight, or fat content in populations of mice in areas treated with Dimilin 25 WP at a rate of rate of 140 g formulation/ha (35 g a.i./ha). Mice in the treated areas did consume less lepidopteran prey, secondary to the toxicity of diflubenzuron to lepidoptera, but total food consumption was not significantly different in treated and untreated plots.

4.1.2.2. Birds – A relatively large number of acute and subchronic toxicity studies are available in standard test species – i.e., mallard ducks and bobwhite quail – as well as other less commonly tested species – i.e., domestic hens and red-winged blackbirds (Appendix 4). Most of these studies were submitted to the U.S. EPA for the registration diflubenzuron (specified in Appendix 4 by MRID numbers) but some have been published in the open literature (e.g., Kubena 1981,1982, Kubena and Witzel 1980).

The acute toxicity of diflubenzuron to birds appears generally to be low and consistent with the gavage studies in rats in which gavage oral LD_{50} values are greater than 5000 mg/kg (Section 3.1 and Appendix 1). As summarized in Appendix 4, red-winged blackbirds appear to be somewhat more sensitive than mallard ducks – i.e., a gavage NOEL for red-winged blackbirds of 2500 mg/kg compared to a gavage NOEL for mallards of 5000 mg/kg. Nonetheless, diflubenzuron is classified a "virtually non-toxic" to both species as well as to bobwhite quail (U.S. EPA 1997a, p. 44). Based on the results of several standard reproduction studies, the the chronic dietary NOEC in birds is 500 ppm (U.S. EPA/OPP 1997a).

There is one atypical report of adverse reproductive effects in birds. Smalley (1976) reports that Dimilin (NOS), incorporated into the feed (dose not specified) of chicks (presumably chickens) for 13 weeks, resulted in an increased incidence of fat deposition in female chicks. The treated chicks weighed 6 ½ lbs, compared to normal weight of 3 lbs for controls (broilers) and males. In addition, Smalley (1976) reports a dose-related decrease in testosterone in treated males resulting in undeveloped combs, wattles, feathers, and voice. Very few experimental details are included in this study. Given the large number of other studies in birds in which no effects on reproduction were apparent, the report by Smalley (1976) appears to be an aberration.

The lack of direct effects on birds is supported by several field studies summarized in Appendix 3a. Some effects secondary to reduced lepidoptera prey may include increased foraging range (Cooper et al. 1990), relocation (Sample et al. 1993a,b) and lower body fat (Whitmore 1993).

4.1.2.3. Terrestrial Invertebrates – A large and relatively complex body of information is available on the toxicity of diflubenzuron to both target and non-target invertebrates. This information consists of both laboratory studies in which exposures are relatively well defined and controlled (Appendix 5) as well as field studies in which exposures are typically characterized as application rates (Appendix 3a).

A synopsis of the field studies in which exposures can be expressed in units of application rate (g/ha) are presented in Table 4-1. The first column in this table gives ranges of application rates spanning over an order of magnitude. The second and third columns provide species or groups of species in which no adverse effects (column 2) or adverse effects (column 3) were noted within the corresponding range of application rates. For each species or group the reference is given to a field study summarized in Appendix 3a. A similar summary table is not provided for the laboratory toxicity studies. As discussed further in the dose-response assessment

(Section 4.3.2.3), these studies were conducted using highly variable experimental designs and meaningful comparisons among the various toxicity assays summarized in Appendix 5 are difficult. Additional details of the comparisons among the various field studies are also provided in the dose-response assessment (see discussion of Table 4-5 in Section 4.3.2.3).

The insecticidal action of diflubenzuron is based on the inhibition of chitin synthesis. Chitin is a polymer (repeating series of connected chemical subunits) of a glucose-based molecule and comprises a substantial proportion of the exoskeleton (outer-shell) of insects. Consequently, the inhibition of chitin synthesis disrupts the growth and development of insects. Chitin is also contained in other arthropods (i.e., crustaceans, spiders, and centipedes) as well as some fungi. Thus, the mode of action of diflubenzuron as a insecticide to target species is also relevant to effects on non-target insects as well as other arthropods (Cardona 1999; Cunningham 1986; Eisler 1992; Fisher and Hall 1992; Hobson 2001; Lengen, 1999; Wilson 1997; Wilcox and Coffey 1978). Diflubenzuron also exerts ovicidal effects in several species (Ables et al. 1977; Büchi and Jossi, 1979; Kumar et al. 1994;) and has been shown to inhibit egg production in some species (Rumpf et al. 1998; Medina et al. 2002; Medina et al. 2003).

While the mechanism of action of diflubenzuron is not specific to target insects, there is ample data indicating substantial differences in sensitivity among various groups of terrestrial invertebrates. Invertebrates without exoskeletons, such as earthworms and snails, do not utilize chitin and diflubenzuron is relatively non-toxic to these species (Berends and Thus 1992; Berends et al. 1992). Even among different groups of arthropods, however, differences in sensitivity to diflubenzuron seem apparent. Species that are most sensitive to diflubenzuron include lepidopteran and beetle larvae, grasshoppers and other chewing herbivorous insects (Berry et al. 1993; Butler 1993; Butler et al. 1997; Elliott and Iyer 1982; Jepson and Yemane 1991; Jepson and Martinat et al. 1998, 1993; Kumar et al. 1994; McWhorter and Shapard 1971; Sample et al.1993b; Sinha et al. 1990; Redfern et al. 1980; Yemane 1991). Other species are relatively tolerant to diflubenzuron. These include flies, wasps that are parasites on insect eggs, adult beetles, and sucking insects (Ables et al. 1975; Broadbent and Pree, 1984a; Brown and Respicio, 1981; Bull and Coleman, 1985; De Clercq et al. 1995b; Deakle and Bradley 1981; Delbeke et al. 1997; Gordon and Cornect, 1986; Keever et al. 1977; Martinat et al., 1988; Webb et al. 1989; Zacarias et al. 1998; Zungoli et al. 1983).

The honey bee is a standard test species used by U.S. EPA to classify the toxicity of pesticides to non-target invertebrates. Based on early acute oral and contact toxicity studies in honey bees with LD_{50} values of >30 µg/bee and >114.8 µg/bee (Atkins et al. 1974; Stevenson 1978), the U.S. EPA (1997a) has classified diflubenzuron as *"practically non-toxic to honey bees"* (U.S. EPA 1997a, p. 81). As discussed further in the dose-response assessment (Section 4.3.2.3), several other laboratory toxicity studies also indicate that diflubenzuron is not highly toxic to bees (Chandel and Gupta 1992; Elliott and Iyer, 1982; Gijswijt, 1978; Kuijpers, 1989; Nation et al. 1986; Yu et al. 1984) and this is supported for several field studies conducted at application rates comparable to or substantially higher than those used to control the gypsy moth (Buckner et al. 1975; Emmett and Archer 1980; Matthenius, 1975; Schroeder 1978a; Schroeder 1980). In

addition, no detectable amounts of diflubenzuron were found in honey bees in areas treated with diflubenzuron (Cochran and Poling 1995). Some studies have noted adverse effects in bees. As summarized in Appendix 5, Stoner and Wilson (1982) and (Thompson and Wilkins 2003) noted transient decreases in brood production at relatively high concentrations (10 ppm) in longer term exposures. At 1 ppm or less, however, no effects were noted. Barrows (1995) noted a decrease in the mean number of pollinating insects in watersheds during a year in which diflubenzuron was applied but not in the following year.

In addition to the acute toxic effects of diflubenzuron, mediated primarily through inhibition of chitin, adverse reproductive effects have been reported in several different orders of insects including moths (Beevi and Dale 1984; Tembhare and Shinde 1998), beetles (Büchi and Jossi 1979; Khebbeb et al. 1997; Mani et al. 1997; Soltani and Soltani-Mazouni 1994a,b,1995a,b,1997), grasshoppers (Mathur 1998), lacewings (Medina et al. 2002; Medina et al. 2003; Rumpf et al. 1998), and true bugs – i.e., Order Hemiptera including the suborder Heteroptera (Redfern et al. 1980; Sindhu and Muraleedharan 1997).

In Lepidoptera, reproductive effects were reported by Beevi and Dale (1984), who noted a high incidence of sterility in the rice swarming caterpillar (*Spodoptera mauritania*) after exposures to relatively high concentrations of Dimilin – 10 ppm and higher. The mechanism of this reproductive effect is unclear but may involve the endocrine system – i.e., hormone release by neurosecretory cells. This has been noted in larvae of the fruit-sucking moth, *Othreis materna* (Tembhare and Shinde 1998) and in the cotton bug (*Dysdercus cingzrlattis*) (Sindhu and Muraleedharan 1997). In some other species of Lepidoptera – i.e., tufted apple bud moth – pupae are sensitive to diflubenzuron but no effects are apparent on reproduction (Biddinger and Hull 1999).

In beetles (Coleoptera), effects on larvae, eggs, and reproductive performance have been noted (Büchi and Jossi1979; Mani et al. 1997). In the mealworm, diflubenzuron impacts lipid metabolism in fat bodies and ovaries (Khebbeb et al. 1997). A series of studies in this species (Soltani and Soltani-Mazouni 1997; Soltani-Mazouni and Soltani1994a,b, 1995b) suggest that the decreased fecundity observed in this and other insect species may be associated with the effect of diflubenzuron on oogenesis, possibly due to changes vitellogenic precursors, the production of ecdysteroid by follicle cells, and/or the inhibition of ovarian DNA synthesis. Direct damage to ovary tissue has also been observed in one species of Orthoptera, a grasshopper, but the mechanism of action in this species has not been studied (Mathur 1998).

Reproductive effects in lacewings (Neuroptera) have been noted by Rumpf et al. (1998) and Medina et al. (2002, 2003). As detailed in Appendix 5, contact exposures to diflubenzuron at 0.07 $\mu g/cm^2$ resulted in a substantial decrease in egg production and complete infertility in 13% of the exposed animals. No effects on egg production or hatching in this species have been observed after direct topical applications at doses as low as 0.5 ng/insect. At a substantially higher dose, 75 ng/insect, egg hatching was reduced by 32%. (Medina et al. 2002, 2003).

4.1.2.4. Terrestrial Plants (Macrophytes) **–** As noted in U.S. EPA/OPP (1997a), no terrestrial plant toxicity studies had been submitted to the U.S. EPA at the time of the reregistration of diflubenzuron. In the literature search conducted for the current risk assessment, no bioassays for herbicidal activity of diflubenzuron were encountered in either the published literature or in the more recent U.S. EPA/OPP files.

There are a large number of terrestrial field studies regarding the efficacy of diflubenzuron applied to terrestrial vegetation for the control of various insect pests including the gypsy moth (Appendix 3a). If diflubenzuron were toxic to terrestrial plants at application rates that are used in the field, it is plausible that adverse effects would have been reported in this literature. No such reports were encountered. Thus, there is no basis for asserting that diflubenzuron will cause adverse effects in terrestrial plants and such effects will not be considered quantitatively in this risk assessment.

4.1.2.5. Terrestrial Microorganisms **–** As discussed in Section 3.2 and summarized in Appendix 2 (Environmental Fate) and Appendix 3a (Terrestrial Field Studies), diflubenzuron is readily degraded by terrestrial microorganisms. The degradation of diflubenzuron by soil microorganisms suggests that this compound is not toxic to soil microorganisms and this presumption may account for the relatively few studies on microbial toxicity. Fungi, however, do contain chitin in cell walls and thus could be a potential target. Booth (1978) found no inhibition of fungal growth in several species of fungi (*Aspergillus*, *Fusarium*, *Rhizopus*, *Trichoderma*) at concentrations of up to 100 ppm in growth media – i.e. mg diflubenzuron per kg of soil. Some growth inhibition, however, was noted in a species of *Pythium* at a concentration of 50 ppm. Inhibition of *Rhizoctonia solani*, another terrestrial fungus, has been noted at 300 ppm (Townshend et al. 1983).

The lack of microbial toxicity was also specifically noted in one field study in which no effects on soil or litter populations of bacteria, actynomycetes or fungi were noted after applications of diflubenzuron at a rate of 67.26 g/ha (Kurczewski et al. 1975; Wang 1975), field and laboratory studies on molds and leaf litter or soil bacteria (Landolt and Stephenson 1995), and studies on mycorrhizal or debris decomposing fungi (Iskra et al. 1995; Gundrum et al. 1995).

One study has noted minor and transient changes in microbial activity. Sexstone (1995) conducted a laboratory study in which soil cores were treated at 4.418µg/44.2 cm^2, roughly equivalent to an application rate of 10 g/ha [4.418µg/44.2 cm^2 × 10,000 cm^2/m^2 × 10,000 m^2/ha = 9,995,475 µg/ha ≈ 10 g/ha]. Only transient and sporadic decreases were noted in microbial biomass [Figure 14-1 in Sexton 1995]. These changes in microbial activity were apparent up to day 35 after treatment but there were no changes by 64 days after treatment. Changes in respiration [Figure 14-2 in Sexton 1995] and nitrification [Figures 14-3 to 14-6 in Sexton 1995] and appear to insubstantial. While some of the differences were statistically significant at some time points, Sexstone (1995) characterizes the effects a "minor" and this assessment appears reasonable.

4.1.3. Aquatic Organisms.

4.1.3.1. Fish – The toxicity of diflubenzuron to fish is well characterized in terms of both acute and chronic toxicity and one mesocosm study is available (Appendix 6). In addition, several of the aquatic field studies (Appendix 3b) involve observations on fish populations. Diflubenzuron has a low order of acute toxicity to fish, with 96-hour LC_{50} values in the range of over 25 mg/L(the value for yellow perch reported by Johnson and Finley 1980) to over 500 mg/L (the value for fathead minnow reported by Reiner and Parke 1975). In addition to data on technical grade diflubenzuron, some studies have also been conducted on Dimilin 25W (Julin and Sanders 1978 with additional studies summarized in U.S. EPA 1997a) and these studies indicate that the toxicity of Dimilin 25W is not greater than the toxicity of technical grade diflubenzuron. No studies have been encountered on the acute toxicity of Dimilin 4L to fish. Based on the available information, the U.S. EPA (1997a, p. 47) has classified diflubenzuron as *"practically non-toxic"* to fish in terms of risks from acute exposures.

Diflubenzuron also appears to be relatively non-toxic to fish in longer term exposures. One standard assay for longer term toxicity in fish involves exposing fish eggs to a compound and maintaining the exposure through to the fry stage. In this type of assay, concentrations up to 45 ppb has no effect on egg or fry of steelhead trout, fathead minnows, or guppies (Hansen and Garton 1982a). In addition, no effects were seen in longer-term studies at concentrations up to 100 ppb (Cannon and Krize 1976) or in 2-generation reproduction studies at concentrations of up to 50 ppb (Livingston and Koenig 1977).

As discussed in Section 4.1.3.2, diflubenzuron is much more toxic to invertebrates than to fish and indirect effects on fish are plausible based on a decrease in invertebrate populations. Such effects have been demonstrated in mesocosm studies (Moffett and Tanner 1995; Tanner and Moffett 1995) in which concentrations as low as 2.5 ppb resulted in decreased growth of fish in littoral enclosures – i.e., populations of fish placed and monitored in enclosures along the shore of a body of water. The reduced growth observed in these studies was attributed to a reduction in macroinvertebrates that serve as a food source for the fish.

It is unclear, however, that secondary effects on fish growth or populations will be observed in the field. None of the field studies summarized in Appendix 3b note any adverse effects on fish in applications comparable to or greater than those used in the control of the gypsy moth. For example, Farlow et al. (1978) conducted a relatively large field study in a marsh area treated with six applications of diflubenzuron at 28 g a.i./ha – i.e., a cumulative application of 168 g/ha. While substantial shifts were noted in various invertebrates (Appendix 3a and Section 4.1.3.2), populations of mosquito fish (*Gambusia affinis*) and American flag fish (*Jordanella floridae*) increased. Similarly, no effects on the growth of fish were noted in ponds directly treated with diflubenzuron at a concentration of 5 ppb (Apperson et al. 1977, 1978) or 13 ppb (Colwell and Schaefer 1980). The study by Colwell and Schaefer (1980) did note a shift in diet of fish (secondary to changes in food availability) but no effect on growth rates or general condition of the fish.

4.1.3.2. Amphibians – Amphibians are not standard test organisms for toxicity studies and no standard bioassays on amphibians have been encountered in the open literature or U.S. EPA/OPP files. Two field studies (Pauley 1995a,b), however, are available on salamanders. Both of these studies were conducted as part of a large study on the effects of spraying diflubenzuron in the northeast for control of the gypsy moth (Reardon 1995a). In this study, two watersheds were treated with Dimilin 4L in 1992 at a rate of 80g/ha (0.03 lb/acre) (Reardon 1995b). Pauley (1995a,b) conducted field studies to assess effects on both aquatic (Pauley 1995a) and terrestrial salamanders (Pauley 1995b). While all salamanders are amphibians, some species spend most of their time on land while others spend most of their time in water. In aquatic salamanders, diflubenzuron treatment was associated with a shift in dietary consumption to more hard-bodied prey secondary to a reduction in the availability of soft-bodied prey. This is similar to the pattern with fish as noted above. No effects in salamanders, however, were noted based on body size or population (Pauley 1995). In terrestrial salamanders, similar results were observed with no change in body size or body fat associated with treatment but a shift was seen in food consumption to hard-bodied prey (Pauley 1995b).

4.1.3.3. Aquatic Invertebrates – As summarized in Appendix 7, there is a very large and diverse body of literature indicating that diflubenzuron is highly toxic to many aquatic invertebrates. Because diflubenzuron inhibits the synthesis of chitin, crustaceans (arthropods which rely on chitin synthesis for the formation of the exoskeleton) are the aquatic invertebrates that are most sensitive to diflubenzuron.

One of the most common crustacean species used in freshwater invertebrate toxicity studies is *Daphnia magna*, a member of Daphnidae in the order Cladocera. These and other zooplankton feed on aquatic algae and are a source of food for fish. Many bioassays, both acute and chronic, have been conducted on *Daphnia magna* (Hansen and Garton 1982a; Kuijpers 1988; Majori et al. 1984; Surprenant 1988) as well as a related species, *Ceriodaphnia dubia* (Hall 1986). As detailed further in the dose-response assessment, these organisms are among the most sensitive to diflubenzuron, with acute LC_{50} values of about 2 µg/L (Hall 1986; Hansen and Garton 1982a). Several other crustacean species appear to be about as sensitive or only somewhat less sensitive to diflubenzuron as daphnids (Appendix 7).

Broad generalizations are somewhat difficult to make, however, because of the diversity of the studies that have been conducted. Nonetheless, large insects appear to be much more tolerant to diflubenzuron than crustaceans, with acute LC_{50} values on the order of 2123 µg/L for backswimmers (Lahr et al. 2001) and an NOEC of 250 µg/L for scavenger beetles (Miura and Takahashi 1974).

Organisms that do not rely on chitin for an exoskeleton are much less sensitive to diflubenzuron. In the microcosm study by Corry et al. (1995) concentrations of diflubenzuron that caused adverse effects in cladocerans caused no adverse effects in rotifers – an aquatic invertebrate that lacks an exoskeleton. Similar tolerance in rotifers have been observed in littoral enclosure studies at diflubenzuron concentrations of up to 30 µg/L (Liber and O'Halloran 1995). At about

the same concentration, 30 µg/L, two species of snails and aquatic worms were not affected by exposures to diflubenzuron (Hansen and Garton 1982a,b). One common genus of snail, *Physa*, had a reported LC_{50} value of greater than 125 mg/L – i.e., 125,000 µg/L. Ostracods (small bivalve crustaceans) were not affected by diflubenzuron at concentrations up to 2.5 µg/L (Liber and O'Halloran 1995) and much larger Quahog clams (*Mercinaria mercinaria*) were unaffected at concentrations up to 320 µg/L (Surprenant 1989).

As with fish, no data have been located on the toxicity of Dimilin 4L. Lahr (2000, 2001) used a "solvent based" formulation of diflubenzuron but did not specify the formulation as Dimilin 4L. The 48-hour EC_{50} of 0.74 µg/L (0.60-0.88 µg/L) of the solvent based formulation in fairy shrimp, *Streptocephalus sudanicus* reported by Lahr (2001) is comparable to EC_{50} value of 0.65 µg/L for technical grade diflubenzuron reported in grass shrimp, *Palaemonetes pugio* (Tourat and Rao 1987). Toxicity studies are available on Dimilin 25W and, as with fish, the toxicity of Dimilin 25W appears to be the same as technical grade diflubenzuron when exposures are expressed in units of active ingredient (Wilson and Costlow 1986). Thus, there does not appear to be a basis for asserting that the formulated products containing diflubenzuron are more hazardous than diflubenzuron itself.

The available field studies on the effects of diflubenzuron on aquatic invertebrates reenforce the standard toxicity studies, indicating that diflubenzuron will impact invertebrate populations. Several of these studies, however, were conducted at application rates substantially higher than those used to control the gypsy moth. As noted in the program description (Section 2), the maximum application rate that will be used in USDA programs is about 70 g/ha. Many of the studies in which severe adverse effects were observed in aquatic invertebrate populations involved multiple applications at rates between about 110 g/ha and 560 g/ha (e.g., Ali and Mulla 1978a,b; Ali et al. 1988; McAlonan 1975). Similarly, other field studies involve direct applications to open water, a treatment method that is not part of USDA program activities, and which resulted in water concentrations that are in the range of 10 ppb (e.g., Apperson et al. 1977; Boyle et al. 1996; Colwell and Schaefer 1980; Lahr et al. 2000; Sundaram et al. 1991). As discussed further in Section 4.2, concentrations of 10 ppb or greater are in the range of peak concentrations that are likely to be encountered in USDA programs. Concentrations in the range of 10 ppb, however, are substantially higher than average concentrations of diflubenzuron in water that are likely to be encountered in USDA programs.

Those field studies that used lower application rates more typical of USDA programs (e.g., Farlow 1976; Griffith et al. 1996; Griffith et al. 2000; Hurd et al. 1996; Jones and Kochenderfer 1987; Reardon 1995a) have noted some effects on freshwater invertebrates, particularly smaller crustaceans, but the effects were much less severe than those seen in the higher application rate studies. This is discussed further in Section 4.4 (Risk Characterization).

4.1.3.4. Aquatic Plants – Data on the toxicity of diflubenzuron to aquatic plants is summarized in Appendix 8. Most studies report no direct toxic effects of diflubenzuron on aquatic plants (algae or macrophytes) at concentrations of 100 µg/L or higher (Booth and Ferrell 1977;

Thompson and Swigert 1993a,b,c) and no indirect effects on aquatic macrophytes (Moffett 1995). A decrease in periphyton in littoral enclosures, however, was noted by Moffett (1995) at 7.0, or 30 µg/L but not at 0.7 or 2.5 µg/L. This effect was attributed not to a direct toxic effect on the periphyton but to the loss of grazers (e.g., cladocera) that may have induced premature senescence in periphyton secondary to a decrement in water quality.

4.1.3.5. Aquatic Microorganisms – There is very little information suggesting that diflubenzuron will adversely affect aquatic microorganisms. No marked differences in numbers of fungal taxa in treated and untreated watersheds were noted by Dubey (1995) in a survey of watersheds treated with diflubenzuron for the control of the gypsy moth. In an aquatic mesocosm, Kreutzweiser et al. (2001) did note a slight but significant effect of diflubenzuron (50 µg/L and 50,000 µg/L) on microbial decomposition and respiration. Changes at 50 µg/L, however, were only marginally significant and variable over the 21-day period.

In the Kreutzweiser et al. (2001) study, Dimilin 4L was used. This is the only laboratory study involving Dimilin 4L. Because no corresponding studies are available on Dimilin 25W or technical grade diflubenzuron, inferences concerning the potential effect of the petroleum solvent in Dimilin 4L cannot be made.

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview

As in the human health risk assessment (Section 3.2), exposures are estimated for both diflubenzuron and 4-chloroaniline. A full set of exposure assessments are developed for diflubenzuron but only a subset of exposure assessments are developed for 4-chloroaniline. This approach is taken, again as in the human health risk assessment, because 4-chloroaniline is assessed as an environmental metabolite of diflubenzuron. Thus, immediately after application, the amount of 4-chloroaniline as an environmental metabolite will be negligible. Consequently, the direct spray scenarios as well as the consumption of insects and the consumption of small mammals after a direct spray are not included for 4-chloroaniline. Also as in the human health risk assessment, all standard chronic exposure scenarios are included for 4-chloroaniline. Details of the exposure assessments for diflubenzuron and 4-chloroaniline are given in the two sets of worksheets that accompany this risk assessment: Supplement 1for diflubenzuron and Supplement 2 for 4-chloroaniline. All exposure assessments are based on the maximum application rate of 70 g/ha.

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 diflubenzuron, the highest acute exposures for small terrestrial vertebrates will occur after a direct spray and could reach up to about 10 mg/kg at an application rate of 70 g/ha. Exposures anticipated from the consumption of contaminated vegetation by terrestrial animals range from central estimates of about 0.08 mg/kg for a small mammal to 2 mg/kg for a large bird with upper ranges of about 0.2 mg/kg for a small mammal and 5 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 a small mammal from the consumption of contaminated vegetation at the application site are in the range of about 0.001 mg/kg to 0.005 mg/kg. Large birds feeding on contaminated vegetation at the application site could be exposed to much higher concentrations, ranging from about 0.08 mg/kg/day to 0.7 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 0.0000001 mg/kg/day to 0.00001 mg/kg/day for a small mammal.

Exposures of terrestrial organisms to 4-chloroaniline tend to be much lower than those for diflubenzuron. The highest acute exposure 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 is 0.0002 mg/kg/day, the dose associated with the consumption of contaminated vegetation by a large bird.

Exposures to aquatic organisms are based on the same information used to assess the exposures of terrestrial species from contaminated water. At the maximum application rate of 70 g/ha, the upper range of the expected peak concentration of diflubenzuron in surface water is taken as 16 µg/L. The lower range of the concentration in ambient water is estimated at 0.01 µg/L. The central estimate of concentration of diflubenzuron in surface water is taken as 0.4 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 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 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 mammals involve the consumption of contaminated

insects by a small mammal (Worksheet F14a) and the consumption by a large mammalian carnivore of small mammals contaminated by direct spray (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 of contaminated grasses by a large bird (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 diflubenzuron 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 a steady state 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 diflubenzuron indicates that this compound will accumulate in the tissue of the fish. Thus, it is plausible that absorbed dose resulting from contact with contaminated vegetation will be as great as those associated with comparable direct spray scenarios.

4.2.2.3. Ingestion of Contaminated Vegetation or Prey – Since diflubenzuron 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).

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 B21). 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, such as a deer. 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 Worksheet 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 that100% of the animals 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 (lowering 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 B24 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 on vegetation are based on the relationship between application rate and residue rates on different

types of vegetation. As summarized in Worksheet B21, 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 diflubenzuron in insects after applications of diflubenzuron. 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 F16a) or a predatory bird (Worksheet F16b). Each of these scenarios assumes that the small mammal is directly sprayed at the specified application rate 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, diflubenzuron 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 diflubenzuron in water are identical to those used in the human health risk assessment (Worksheet A04). 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 variability of the ingested dose estimates 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 diflubenzuron. 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 diflubenzuron, 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 diflubenzuron 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, 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 4-2. As indicated in this table, peak soil concentrations at an application rate of 70 g/ha are in a relatively narrow range: about 0.003 to 0.009 mg/kg (ppm) over all soil types and rainfall rates. Longer term concentrations in soil are all low and are on the order of 0.00005 to 0.0005 mg/kg – i.e., 0.05 ppb to 0.5 ppb. Modeled concentrations of 4-chloroaniline in soil are summarized in Table 4-3. As would be expected of any environmental metabolite, peak concentrations are lower than those of the parent compound. For 4-chloroaniline these range from about 0.0007 to 0.003 mg/kg, about a factor of three lower than the corresponding concentrations of diflubenzuron.

4.2.5. Aquatic Organisms

The potential for effects on aquatic species are based on estimated concentrations of diflubenzuron and 4-chloroaniline in water that are identical to those used in the human health risk assessment. As summarized in Table 3-8, the peak estimated concentration of diflubenzuron in ambient water is 0.4 (0.01 to 16) µg/L at an application rate of 70 g/ha. For longer-term exposures, the corresponding longer term concentrations in ambient water are estimated at 0.02 (0.001 to 0.1) µg/L. The corresponding estimates for 4-chloroaniline are summarized in Table 3-9: 0.5 (0.00003 to 2) µg/L for acute exposures and 0.05 (0.0002 to 0.2) µg/L for longer term exposures.

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Overview

As in the human health risk assessment, toxicity values are derived for both diflubenzuron and 4-chloroaniline. Several of the toxicity values used in the ecological risk assessment for diflubenzuron are summarized in Table 4-4. For two groups of organisms, terrestrial arthropods and aquatic invertebrates, detailed dose-response assessments can be made for several different subgroups. These toxicity values are summarized in Table 4-5 for terrestrial arthropods and Table 4-6 for aquatic invertebrates. The values for 4-chloroaniline are summarized in Table 4-7.

Diflubenzuron 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 of 1118 mg/kg and a chronic NOAEL of 2 mg/kg/day. A similar approach is taken for 4-chloroaniline for which an acute NOAEL of 8 mg/kg is used based on a subchronic study and a chronic NOAEL is estimated at 1.25 mg/kg/day based on the chronic LOAEL of 12.5 mg/kg/day. For birds, the acute NOAEL for diflubenzuron is taken as 2500 mg/kg from an acute gavage study and the longer term NOAEL is taken as 110 mg/kg/day from a reproduction study. No data are available on toxicity of 4-chloroaniline in birds and the available toxicity values for mammals are used as a surrogate.

For terrestrial invertebrates two general types of data could be used to assess dose-response relationships: laboratory toxicity studies and field studies. Field studies are used in the current risk assessment 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 appear 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 22 g/ha. Somewhat high application rates – in the range of 30 to 35 g/ha – will adversely affect macrolepidoptera and some beneficial parasitic wasps. At the maximum application rate considered in this risk assessment – i.e., 70 g/ha – some 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 400 g/ha.

Invertebrates that do not utilize chitin are also relatively insensitive to diflubenzuron. The NOEC for a species of earthworm (*Eisenia fetida*) is 780 mg/kg soil and is used to represent tolerant species of soil invertebrates. Very little information is available on the toxicity of 4-chloroaniline to terrestrial invertebrates. As with diflubenzuron, the earthworm appears to be relatively tolerant to 4-chloroaniline with a reported LC_{50} value of 540 mg/kg dry soil. The toxicity of both diflubenzuron and 4-chloroaniline to soil microorganisms is also relatively low.

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. For

diflubenzuron, LC_{50} values of 25 mg/L to 500 mg/L are used to characterize risks for sensitive and tolerant species of fish, respectively. 4-Chloroaniline appears to be more toxic to fish and an LC_{50} value of 2.4 mg/L is used to characterize risks of peak exposures and 0.2 mg/L is used to characterize risks of longer term exposures.

Substantial variability in the response of different groups of aquatic invertebrates to diflubenzuron is apparent. Very small arthropods appear to be among the most sensitive species – with acute NOEC values in the range of 0.3 to about 1 ppb (µg/L) and chronic NOEC values in the range of 0.04 to 0.25 ppb. Based on acute NOEC values, larger arthropods, including crabs and larger insects, appear to be more tolerant, with acute NOEC values in the range of 2 to 2000 ppb. For chronic effects, the differences between small and larger arthropods are less remarkable, a stoneflies and mayflies (relatively large insects) having an NOEC value of 0.1 ppb, intermediate between *Daphnia* (0.04 ppb) and *Ceriodaphnia* (0.25 ppb). Molluscs (invertebrates including clams and snails) and worms (oligochaetes) appear to be much less sensitive to diflubenzuron.

The data on the toxicity of 4-chloroaniline to aquatic invertebrates is sparse. An acute NOEC of 0.013 mg/L is used to characterize acute risks associated with peak exposures in aquatic invertebrates and an NOEC of 0.01 mg/L from a reproduction study is used to characterize longer term risks to aquatic invertebrates.

4.3.2. Toxicity to Terrestrial Organisms

4.3.2.1. Mammals – The dose-response assessment for mammalian wildlife species is based on the same set of studies used in the human health risk assessment for diflubenzuron (Section 3.3.2) and 4-chloroaniline (Section 3.3.3).

For diflubenzuron, the most sensitive effect in experimental mammals involves toxic effects in red blood cells. The NOAEL for this endpoint in experimental mammals is 2 mg/kg/day (U.S. EPA 1997a) and is based on a study in which dogs were administered doses of 0, 2, 10, 50, or 250 mg/kg/day, 7 days/week, for 52 consecutive weeks in gelatin capsules (Greenough et al. 1985). No adverse effects, including changes in methemoglobin formation, were noted at 2 mg/kg/day. This dose will be used to characterize longer term risks to mammals. For acute exposures, the acute NOAEL of 1118 mg/kg is used. As discussed in Section 3.3.2.2, this is based on a study using a petroleum based formulation of diflubenzuron, Dimilin 2L. Because none of the estimated exposures approach a level of concern, no elaboration of the dose-response assessment is needed.

A similar approach is taken for 4-chloroaniline. The acute NOAEL is taken as 8 mg/kg. This is a very conservative approach – i.e., likely to be overly protective – because this NOAEL is from a 90 day study (Scott and Eccleston 1967). The chronic value is based on a LOAEL of 12.5 mg/kg/day from a 2-year feeding study using rats (NCI 1979). Because a NOAEL was not identified in this study, the LOAEL of 12. 5 mg/kg/day is divided by 10 to estimate a chronic

NOAEL of 1.25 mg/kg/day. This is essentially the same estimate used by U.S. EPA (1997a) in the derivation of the RfD based on the LOAEL of 12.5 mg/kg/day (Section 3.3.3.1).

4.3.2.2. Birds

4.3.2.2.1. Diflubenzuron – There appears to be relatively little difference in the acute toxicity of diflubenzuron to birds and mammals. As summarized above, the lowest acute NOAEL for mammals is 1118 mg/kg (rats dosed with Dimilin 2L in the study by Blaszcak (1997a). For birds, the lowest acute NOAEL is 2500 mg/kg from the study by Alsager and Cook (1975) in red-winged blackbirds. As detailed in Appendix 1 for mammals and Appendix 8 for birds, higher NOAEL values have been reported in other studies – i.e., up to 10,000 mg/kg for mammals (rats and mice in the study by Koopman 1977) and 5,000 mg/kg for birds (mallard ducks in the study by Roberts and Parke 1976). Analogous to the approach taken with rats, the lowest NOAEL is taken as the toxicity value for acute exposures in bird – i.e., the NOAEL of 2500 mg/kg in red-winged blackbirds from the study by Alsager and Cook 1975.

It should be noted that the variability in the acute NOAEL values does not imply any systematic differences among species but simply reflects the highest dose tested in the different experiments. Thus, the use of the lowest NOAEL rather than the highest NOAEL may be viewed as somewhat conservative. As discussed in Section 4.3.2.1, the use of the 1118 mg/kg dose for mammals is justified based on the use of a petroleum based formulation in the study by Blaszcak (1997a). The use of the lowest NOAEL for birds based on the conservative assumption that somewhat higher doses in the study by Alsager and Cook (1975) could have resulted in effects. Notwithstanding this assumption, the data are not sufficient to derive separate NOAEL values for tolerant and sensitive species because none of the available data actually demonstrated differences in sensitivity – i.e., differences in LOAEL values.

In terms of chronic toxicity, however, birds appear to be somewhat more tolerant to diflubenzuron than mammals. Based on reproduction studies, the NOEC for reproductive toxicity in birds is greater than 500 ppm – i.e., at the highest dietary concentration, no effects were noted – in mallard ducks (Beavers et al. 1990a) and bobwhite quail (Beavers et al. 1990b). Based on differences in food consumption (Appendix 4), the lowest dose in terms of mg/kg bw/day is 110 mg/kg/day from the study in quail (Beavers et al. 1990b). This is substantially above for the mammalian NOAEL of 2 mg/kg/day and the corresponding mammalian LOAEL of 10 mg/kg/day. While this suggests a difference in sensitivity between mammals and birds, the toxicity endpoints are different – i.e., effects on blood from chronic exposure in mammals and reproductive effects in birds. As noted in Appendix 1, doses as high as about 4000 mg/kg/day were not associated with reproductive effects in rats (Brooker 1995). In any event, the chronic NOAEL of 110 mg/kg/day in quail from the study by Beavers et al. (1990b) is used to characterize the risks associated with longer term exposures of birds to diflubenzuron.

4.3.2.2.2. 4-Chloroaniline – No data have been encountered on the toxicity of 4-chloroaniline to birds. For the current risk assessment, the toxicity values for 4-chloroaniline

in mammals are used as surrogates for birds. This adds uncertainty to the risk assessment for birds and this is discussed further in Section 4.4 (Risk Characterization).

4.3.2.3. Terrestrial Invertebrates

4.3.2.3.1. Diflubenzuron – Two general types of data could be used to assess dose-response relationships for terrestrial invertebrates: laboratory toxicity studies (Appendix 5) and field studies (Appendix 3a). In most risk assessments conducted by U.S. EPA (e.g. U.S. EPA/OPP 1997a) as well as risk assessments conducted for the USDA/Forest Service, dose-response assessments for terrestrial invertebrates are based on controlled laboratory studies that are commonly conducted on the honey bee using relatively standard protocols. As indicated in Table 4-5, a different approach is used in the current risk assessment: the large number of field studies on diflubenzuron that report either effect or no effect levels are used directly for characterizing risk with exposures expressed in units of application rate.

One reason for this approach involves the disparity in experimental designs among the toxicity studies that are available which confounds quantitative comparisons of relative sensitivities among species. As discussed in Section 4.1.2.3, there is an apparently wide range of sensitivities to diflubenzuron among different invertebrate species. Based on standard toxicity tests, the honey bee is among the more tolerant species. The U.S. EPA used an LD_{50} of greater than 30 µg/bee to classify diflubenzuron as practically non-toxic to the honey bee. Taking an average weight of 0.093 g/bee or 0.000093 kg/bee (USDA/APHIS 1993) and making the very conservative assumption of 100% absorption, this would correspond to an LD_{50} greater than 322 mg/kg bw [0.03 mg/bee ÷ 0.000093 kg bw/bee = 322.58 mg/kg]. As summarized in Appendix 5, somewhat lower LD_{50} values have been reported by Chandel and Gupta (1992) – i.e., about 22 mg/kg for pupae and 53 mg/kg for third instar larvae. The gypsy moth is obviously a sensitive species, with a topical LD_{50} value of about 4 to 9 mg/kg, based on residues on vegetation (Berry et al. 1993), about a factor of 2 to 5 below the lowest LD_{50} value for the honey bee. A similar topical LD_{50} of 1.07 mg/kg has been reported by Sinha et al. (1990) for the butterfly, *Pieris brassicae*. Somewhat lower LD_{50} values have been reported for an orthopteran – i.e., 0.31 mg/kg in *Oxya japonica* from the study by Lim and Lee (1982). Based on topical LD_{50} values, the most sensitive species appears to be lacewing, *Chysoperla carnea*, with a reported topical LD_{50} values of 2.26 ng/insect or about 0.00226 µg/insect (Medina et al. 2003). Based on a mean body weight of 7.53 mg reported by Medina et al. (2003), this corresponds to a dose of 0.0003 µg/mg, which in turn corresponds to a dose of 0.0003 mg/g or 0.0000003 mg/kg bw. Thus, based on this LD_{50}, the lacewing would appear to be more sensitive than the gypsy moth by a factor of 13 to 30 million [4 to 9 mg/kg ÷ 0.0000003 mg/kg]. The LD_{50} value from Medina et al. (2003), however, is not really comparable to the value for the gypsy moth because the topical application to the lacewing involved direct application of diflubenzuron (in acetone) rather than a spray or contact with a contaminated surface. Thus, while the various laboratory toxicity studies could be used to construct a standard dose-response assessment for tolerant and sensitive species, there would be substantial uncertainty in the comparisons because of the diversity in experimental designs.

An alternative approach may be based on the available field studies. A summary of these studies is presented in Table 4-1 and additional details are provided in Appendix 3a. Field studies, like epidemiology studies, can be difficult to interpret because of differences in the treated site versus the control site. For example, the study by Van Den Berg (1986) on mites and collembolans is noted in Table 4-1 as providing a NOAEL in which transient or equivocal effects were noted. As detailed in Appendix 3a, Van Den Berg (1986) concluded that the effects on the mites and collembolans were insubstantial. The data, however, indicate generally fewer species over time in the treated site versus the untreated site. The author's conclusion that the effects were insubstantial is based on the fact that the populations of mites and collembolans were different at the control and treated sites prior to treatment and that the capture patterns over time for mites were highly erratic. In other words, compared to pre-treatment populations as well as the time course of population changes, the effect of diflubenzuron in this study appeared to be marginal and insubstantial. An examination of the data presented by Van Den Berg (1986) supports the conclusion that the application of diflubenzuron in this study should be classified as a NOAEL. A similar assessment may be made of the study by Martinat et al. (1993) in which changes in populations of spiders and orthopteroids (i.e., cockroaches, mantises, locusts, and crickets) were only sporadically noted over time and no consistent effect is apparent.

Despite the difficulty and uncertainty in interpreting some of the fields, the relatively large number of field studies on diflubenzuron appear 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. Consistent with the laboratory studies, the field studies clearly indicate that honey bees are relatively insensitive to diflubenzuron: application rates of up to 400 g/ha are not likely to affect honeybees (Table 4-5). The most sensitive species appear to be grasshoppers which may be adversely affected at an application rate of 22 g/ha. Somewhat high application rates – in the range of 30 to 35 g/ha – will adversely effect macrolepidoptera and some beneficial parasitic wasps. At the maximum application rate of considered in this risk assessment – i.e., 70 g/ha – some 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, as detailed in Table 4-5.

As also noted in Section 4.1.2.3, invertebrates that do not utilize chitin are relatively insensitive to diflubenzuron. Based on soil toxicity studies, the NOEC 780 mg/kg soil for the earthworm (*Eisenia fetida*) from the study by Berends et al. (1992) is used to represent tolerant species of soil invertebrates.

4.3.2.3.2. 4-Chloroaniline – Very little information is available on the toxicity of 4-chloroaniline to terrestrial invertebrates (WHO 2003). This is not uncommon for compounds that are not used or registered as insecticides. WHO (2003) summarizes a standard OECD study on earthworms in which the 28-day LC_{50} value was 540 mg/kg dry soil. As noted in Section 3.2, this is far higher than any concentrations of 4-chloroaniline that are likely to be found in soil.

4.3.2.4. Terrestrial Plants (Macrophytes) – As discussed in 4.1.2.4 (Hazard Identification for Terrestrial Plants), no toxicity studies have been conducted on terrestrial plants and there is no basis for asserting that adverse effects on terrestrial plants are likely from exposures to either diflubenzuron or 4-chloroaniline. Consequently, no dose-response assessments for terrestrial plants are presented in this risk assessment.

4.3.2.5. Soil Microorganisms

4.3.2.5.1. Diflubenzuron – Diflubenzuron does not appear to be very toxic to soil microorganisms (Section 4.1.2.5). While one study (Sexstone 1995) has noted transient changes in gross microbial biomass and activity at one exposure rate (roughly equivalent to 10 g/ha), no dose-response relationship is demonstrated and the effects, if any, appear to be very minor. Consequently, this study is not used quantitatively in the dose-response assessment for soil microorganisms. For the current risk assessment, bioassays on fungi are used to identify tolerant and sensitive species – a LOEC of 50 ppm in *Pythium* for sensitive species and an NOEC of 100 ppm for tolerant species (*Aspergillus*, *Fusarium*, *Rhizopus*, *Trichoderma*) from the study by (Townshend et al. 1983). If any species of microorganisms are at risk from exposure to diflubenzuron, fungi might be considered the most likely to be susceptible because some fungi utilize chitin in their cell walls. As summarized in Table 4-2, however, the NOEC and LOEC values are several orders of magnitude higher than any plausible soil exposures.

4.3.2.5.2. 4-Chloroaniline – The only information encountered on the microbial toxicity of 4-chloroaniline is an ED_{10} of 1000 ppm for Fe(III) reductions by upper soil (Horizon A) microorganisms (Welp and Brummer 1999). As with diflubenzuron, this concentration is far above plausible levels of soil exposure.

4.3.3. Aquatic Organisms

4.3.3.1. Fish

4.3.3.1.1. Diflubenzuron – The toxicity data on diflubenzuron are sufficient to identify sensitive and tolerant species for both acute and chronic exposures (Table 4-4). For acute toxicity, the lowest and highest LC_{50} values will be used consistent with the data in the risk assessment presented by U.S. EPA/OPP (1997a). The LC_{50} value for sensitive fish species will be taken as 25 mg/L from the study by Johnson and Finley (1980) in yellow perch and the LC_{50} value for tolerant fish species will be taken as 500 mg/L from the study by Reiner and Parke (1975) in fathead minnow. Both of these are very protective values in that both concentrations are actually the highest concentration tested and less than 50% mortality was observed. As discussed further in Section 4.4, this protective approach has no impact on the risk assessment because the anticipated peak exposures to diflubenzuron are far below these concentrations. For longer term exposures, reproductive NOEC values will be used. The range of reported values is relatively narrow: 0.05 mg/L for mummichogs from the study by Livingston and Koenig (1977) to 0.1 mg/L for fathead minnows from the study by Cannon and Krize (1976).

4.3.3.1.2. 4-Chloroaniline – Very little information is available on the toxicity of 4-chloroaniline to fish. As reviewed by WHO (2003), an LC_{50} value of 2.4 mg/L is reported in

bluegills and a reproductive NOEC of 0.2 mg/L in zebra fish is reported in Bresch et al. (1990). These values are used in the current risk assessment for characterizing risks to fish associated with exposures to 4-chloroaniline (Table 4-7).

4.3.3.2. Amphibians – The only information on the toxicity of diflubenzuron to amphibians comes from two field studies conducted by Pauley (1995a,b). As discussed in Section 4.1.3.2, these studies indicate a change in the diet of both terrestrial and aquatic salamanders following an application of diflubenzuron at 80g/ha. This change was secondary to changes in available food items. No data are available on the toxicity of 4-chloroaniline to amphibians. Because of the very low apparent risks to fish (Section 4.4), the limited data on effects of diflubenzuron to amphibians, and the lack of data on the effects of 4-chloroaniline to amphibians, a quantitative dose-response assessment for this group of organisms is not proposed.

4.3.3.3. Invertebrates

4.3.3.3.1. Diflubenzuron – The toxicity values used in this risk assessment for aquatic invertebrates are summarized in Table 4-6, with the top section of this table summarizing acute toxicity values that are used to characterize risks associated with peak exposures and the bottom section of the table summarizing toxicity values used to characterize risks associated with longer term exposures. In all cases, the toxicity values are based on no-observed-effect concentrations (NOECs). This approach is somewhat different from the approach taken by U.S. EPA (1997a), in which toxicity values are based on LC_{50} values but the studies used and basic conclusions of the current risk assessment are similar to those of U.S. EPA (1997a). Diflubenzuron is very highly toxic to some aquatic invertebrates.

As with the acute toxicity to terrestrial invertebrates, the dose-response assessment can be elaborated to include several groups of invertebrates rather than simply sensitive and tolerant species. Supporting information for the acute and chronic toxicity values are given in Table 4-8 and Table 4-9, respectively, and additional information from field studies is summarized in Table 4-10. More detailed summarizes of the acute and chronic toxicity studies are given in Appendix 7 and details of a large number of field studies are given in Appendix 3b.

As summarized in Table 4-6, there is a substantial variability in the response of different groups of aquatic invertebrates to diflubenzuron. Very small arthropods – i.e, cladocerans (*Daphnia* and *Ceriodaphnia*) as well as copepods – appear to be among the most sensitive aquatic species – with acute NOEC values in the range of 0.3 to about 1 ppb (µg/L) and chronic NOEC values in the range of 0.04 to 0.25 ppb. Based on acute NOEC values, larger arthropods, including crabs and larger insects, appear to be more tolerant, with acute NOEC values in the range of 2 to 2000 ppb. In some of these assays of larger invertebrates, the short duration of the assay may be a factor in the apparently greater tolerance of larger invertebrates compared to small invertebrates. For example, Lahr et al. (2001) note that the backswimmers tested in their bioassay evidenced a NOEC of 2000 ppb but that lower NOEC values could have been evident if the organisms had been in a molting stage. This supposition is supported by chronic toxicity data (Table 4-9) in which differences between small and larger arthropods are less remarkable, with stoneflies and

mayflies (relatively large insects) having an NOEC value of 0.1 ppb, intermediate between *Daphnia* (0.04 ppb) and *Ceriodaphnia* (0.25 ppb). In the tests using stonefly and mayflies, response was characterized as an inhibition of emergence rather than pre-emergent mortality. Again, this probably relates to the inhibition of chitin synthesis by diflubenzuron. Molluscs (invertebrates including clams and snails) and worms (oligochaetes) appear to be much less sensitive to diflubenzuron.

Based on acute NOEC values, the range of sensitivities among aquatic invertebrates appears to span a factor of over 400,000 [125,000 ppb in molluscs ÷ 0.3 in *Daphnia* = 416,667] based on acute NOEC values and a factor of 8,000 [320 ppb in molluscs ÷ 0.04 in *Daphnia*] based on longer term NOEC values. These ratios are, at least to some extent, artifacts of experimental design. As summarized in Tables 4-8 and 4-9, acute and chronic NOEC and LOEC values are available for sensitive species such as daphnids. For molluscs, however, only NOEC values are available – i.e., no effects have been demonstrated in these species at the highest concentration tested.

Although there is a large number of field studies available on effects of diflubenzuron on aquatic invertebrates (Appendix 3b), these studies are not directly used in the dose-response assessments. Unlike the case with terrestrial invertebrates, application rates (e.g., g/ha) in aquatic field studies do not provide a uniform basis for comparing exposures among the different studies because the amount of diflubenzuron entering the water may and probably did vary remarkably among the different field studies based on site-specific and meteorological differences among the studies. The magnitude of possible differences is illustrated in Tables 3-2 and 3-3.

Nonetheless, some studies provide information on both application and concentrations in ambient water. An overview of these studies, summarized from Appendix 3b, is given in Table 4-10. As in the tables for standard toxicity studies, Tables 4-8 and 4-9, concentrations are given in braces [] between the species and the citation. Even these concentrations, however, are not readily comparable among studies, with some reported as peak concentrations and others as nominal or average concentrations over a given period. For example, Apperson et al. (1977) conducted a field study in which populations of cladocerans and copepods declined after an application of diflubenzuron to ponds and lakes at nominal concentrations of 2.5, 5, and 10 ppb. Actual monitored concentrations peaked at up to 32.2 ppb, however, and declined rapidly to less than 1 ppb. This type of pattern is typical in field studies in which concentrations will vary substantially both among different studies as well as over time within a single study. This probably accounts for the general pattern of field studies suggesting a higher tolerance in terms of reported concentrations than laboratory studies in which concentrations are better defined and less variable. The field studies summarized in Table 4-10, however, do support the general pattern of species sensitivity noted in the laboratory toxicity studies – i.e., small arthropods are more sensitive than larger arthropods and non-arthropod invertebrates.

Notwithstanding the limitations inherent in field studies in terms of actual exposures and temporal variations, the field studies are directly useful in risk characterization and are discussed

further in Section 4.4. One very important feature of field studies is ability to assess population recovery, which is not typically assayed in laboratory studies. As summarized in Table 4-10, most field studies that detect adverse effects also find evidence of population recovery after application so long as the duration of the study is sufficiently long to permit the detection of recovery. This is also discussed further in the risk characterization (Section 4.4).

4.3.3.3.2. 4-Chloroaniline – The data on the toxicity of 4-chloroaniline to aquatic invertebrates is sparse, particularly when compared to the very rich data base on diflubenzuron. Notwithstanding this limitation, 4-chloroaniline appears to be much less toxic to aquatic invertebrates than diflubenzuron and the magnitude of the difference in potency can be quantified. In terms of acute toxicity to *Daphnia magna*, the 48-hour LC_{50} value for 4-chloroaniline has been reported as 0.31 mg/L (Kuhn et al 1989a), 400 times higher than the LC_{50} values of 0.0007 mg/L to 0.00075 mg/L for diflubenzuron (Corry et al. 1995; Kuijpers 1988; Majori et al. 1984). The corresponding NOEC for 4-chloroaniline is 0.013 mg/L (Kuhn et al 1989a), 40 times higher than the acute NOEC of 0.0003 mg/L for diflubenzuron (Corry et al. 1995).

Similarly, the chronic NOEC in *Daphnia magna* for 4-chloroaniline in a standard reproduction study is 0.01 mg/L (Kuhn et al 1989b). This is a factor of 250 times higher than the corresponding value of 0.00004 mg/L in *Daphnia magna* reported by Surprenant (1988).

As summarized in Table 4-7 (toxicity values for 4-chloroaniline), the acute NOEC of 0.013 mg/L (Kuhn et al 1989a) is used to characterize acute risks to aquatic invertebrates and the NOEC of 0.01 mg/L for reproductive effects (Kuhn et al 1989b) is used to characterize longer term risks to aquatic invertebrates.

4.3.3.4. Aquatic Plants

4.3.3.4.1. Diflubenzuron – Compared to aquatic invertebrates, relatively little information is available on the toxicity of diflubenzuron to aquatic plants (Section 4.1.3.4 and Appendix 8). The lowest reported effect is a decrease in periphyton at a concentration 7.0 µg/L in littoral enclosures (Moffett 1995). As noted in Section 4.1.3.4 and Appendix 8, Moffett (1995) attributed this change to a decrease in the population density of zooplankton grazers. This conclusion seems reasonable and is supported by standard plant toxicity studies reporting no effects at concentrations of up to 380 µg/L (Booth and Ferrell 1977; Thompson and Swigert 1993a,b,c). For assessing the risks of direct toxic effects on terrestrial plants, a NOEC of 45 µg/L will be used for possibly sensitive species (*Selenastrum capricornutum* in the study by Hansen and Garton 1982a) and a NOEC of 380 µg/L (*Navicula pelliculosa* in the study by Thompson and Swigert 1993c) will be used for apparently tolerant species. Since no LOEC values are available for any species of aquatic plants, these different NOEC values may simply reflect differences in the highest dose tested in the respective experiments rather than true differences in species sensitivity to diflubenzuron.

4.3.3.4.2. 4-Chloroaniline **–** The only information encountered on the toxicity of 4-chloroaniline is summarized in WHO (2003) from two publications in the German literature (Schmidt 1989; Schmidt and Schnabl 1988). Based on this information, 4-chloroaniline appears to be somewhat more toxic to aquatic plants than diflubenzuron. While WHO (2003) does not report NOEC values for 4-chloroaniline, an EC_{10} of 0.02 mg/L for cell multiplication in *Scenedesmus subspicatus*, a species of green algae, will be used as surrogate NOEC.

4.3.3.5. Microorganisms (excluding algae)

4.3.3.5.1. Diflubenzuron **–** Very little information is available on the toxicity of either diflubenzuron or 4-chloroaniline to aquatic microorganisms. As summarized in Section 4.1.3.5, marginal and transient effects on microbial decomposition and respiration have been noted at 50 µg/L and 50,000 µg/L (Kreutzweiser et al. 2001). Because of the insubstantial nature of the effects and the lack of a marked dose-response relationship, the concentration of 50 µg/L is used as a NOEC for aquatic microorganisms in Table 4-4.

4.3.3.5.2. 4-Chloroaniline **–** The only information on 4-chloroaniline is the results of a assay for bioluminescence with *Photobacterium phosphoreum* in which the 30-minute EC_{50} for the inhibition of bioluminescence was 5.1 mg/L (Ribo and Kaiser 1984). While the utility of this type of assay for risk characterization may be marginal, it is the only information available and is included in Table 4-7 and used for the risk characterization of 4-chloroaniline.

4.4. RISK CHARACTERIZATION

4.4.1. Overview

While the data base supporting the ecological risk assessment of diflubenzuron is large and complex, the risk characterization is relatively simple. Diflubenzuron is an effective insecticide and effects on some nontarget terrestrial insects are likely at application rates that are used to control the gypsy moth. Species at greatest risk include grasshoppers, various macrolepidoptera (including the gypsy moth), other herbivorous insects, and some beneficial predators of 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. In areas in which water contamination is likely to be minimal, no or only marginal effects are expected. During applications in which drift or direct deposition is not controlled well or in areas in which soil losses from runoff and sediment are likely, acute effects on some aquatic invertebrates are plausible and longer term effects on sensitive species could occur.

Direct effects of diflubenzuron on other groups of organisms – i.e., mammals, birds, amphibians, fish, terrestrial and aquatic plants, microorganisms, and non-arthropod invertebrates – do not appear to be plausible. Secondary effects in some nontarget species could occur. The most common secondary effects will be seen in and associated with animals that consume either the the gypsy moth or other invertebrates that may be adversely affected by diflubenzuron. The most common secondary effect will be a change in prey items that are consumed. Changes in feeding territory and prey items as well as reductions in body fat are likely to be transient.

There is no indication that 4-chloroaniline formed from the degradation of diflubenzuron will have an adverse effects on any species.

4.4.2. Terrestrial Organisms

4.4.2.1. Terrestrial Vertebrates **–** The risk characterizations for terrestrial vertebrates are essentially identical for both diflubenzuron and 4-chloroaniline. At the highest application rate of diflubenzuron that would be used in USDA programs, risks to mammals and birds are far below a level of concern. The quantitative risk characterization for terrestrial vertebrates (mammals and birds) is summarized in Worksheet G02a in the diflubenzuron worksheets (Supplement 1) and Worksheet G02 in the 4-chloroaniline worksheets (Supplement 2). The risk characterization is based on the estimates of exposure summarized in Section 4.2.3 and the toxicity values for diflubenzuron (Table 4-4) and 4-chloroaniline (Table 4-7) that were derived in Section 4.3.2.

The highest hazard quotient (HQ) for diflubenzuron is 0.2, the value associated with the upper range of exposure from the longer term consumption of contaminated vegetation in the treated area by a large mammal. As discussed in Section 4.2.2, this exposure scenario is based on the consumption of contaminated grass by a large mammal. For the gypsy moth program, this is an

extremely conservative scenario in that most large wildlife mammals will not consume grass as an exclusive or even predominant proportion of their diet (exceptions being elk and some livestock animals). In addition, this scenario assumes that the grass is directly sprayed. In the application of diflubenzuron, canopy interception would reduce residues on grass in most circumstances. Other hazard quotients for diflubenzuron are below a level of concern by factors of 50 (the upper range HQ of 0.02 for the consumption of contaminated fish by a predatory bird) to 1 in one billion (the lower range HQ for the consumption of contaminated water by a small mammal).

The highest risk quotient for chloroaniline is 0.02, associated with the consumption of contaminated water by a small mammal. As discussed in Section 3.2.3.4, these peak exposures may occur months after the application of diflubenzuron and the concentrations of 4-chloroaniline in water are likely to vary substantially with different soils as well as rainfall rates. The peak concentrations of 4-chloroaniline are based on very conservative and perhaps extreme assumptions and the very low of hazard quotient of 0.02 – i.e., below the level of concern by a factor of 50 – indicates that there is no plausible basis for asserting that such exposures would be hazardous.

This risk characterization for terrestrial vertebrates is consistent with the risk characterization by U.S. EPA (1997a) as well as field studies which indicate a lack of adverse effects on terrestrial vertebrates after applications of diflubenzuron (Sections 4.1.2.1 and 4.1.2.2. and Appendix 3a). No toxic effects are likely to be seen in mammals or birds.

The most common secondary effects will be seen in and associated with vertebrates that consume either the target species (the gypsy moth) or other invertebrates that may be adversely affected by diflubenzuron (see Section 4.4.2.2.1). For such vertebrates, the most common secondary effect will be a change in prey items that are consumed.

4.4.2.2. Terrestrial Invertebrates

4.4.2.2.1. Diflubenzuron – While risks to terrestrial vertebrates are implausible, risks to some terrestrial invertebrates are virtually certain (Worksheet G02b, Supplement 1). At an application rate of 70 g/ha, adverse effects – i.e., mortality and decreases in populations – have been demonstrated in field studies for grasshoppers, various macrolepidoptera (including the gypsy moth), some mandibulate herbivores, and some beneficial predators to the gypsy moth. Effects on some beneficial predators may be secondary but at least in one species, *Apanteles melanoscelus*, a wasp that is a parasite on the gypsy moth, the effect appears to be due to direct toxicity (Madrid and Stewart1981). Effects in the same species are likely to be seen at lower application rates that may be used in USDA programs – i.e., 35 g/ha. For effects in these sensitive groups to be avoided, the application rate would need to be below about 2 g/ha [70 g/ha from Worksheet G02b divided by the HQ of 32 for the grasshopper]. This damage to non-target species appears to be unavoidable given 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).

Most other insect groups are not likely to be affected at least directly. Some secondary effects associated with changes in available prey may be noted. As with most secondary effects, the changes in habitat or prey items are likely to be reversible. In other words, changes will be transient and populations will generally recover (e.g., Catangui et al. 1996).

4.4.2.2.2. 4-Chloroaniline – Very little information is available on the toxicity of 4-chloroaniline to invertebrates. One bioassay in earthworms reports an LC_{50} value of 540 mg/kg soil. The maximum concentration of 4-chloroaniline in soil is estimated at 0.0026 ppm (Table 4-3). The resulting HQ is 4.8×10^{-6}, below the level of concern by over 200,000. No data are available on the toxicity of 4-chloroaniline to other terrestrial vertebrates and risks cannot be quantified. Given the relatively low risks of 4-chloroaniline in aquatic invertebrates (4.4.3.2.2) as well as other organisms, there is no basis for asserting that substantial risks are plausible, particularly when compared to clear risks associated with diflubenzuron.

4.4.2.3. Terrestrial Plants and Microorganisms – No quantitative risk assessment to terrestrial plants is made for either diflubenzuron or 4-chloroaniline. As discussed in Section 4.1.2.4, there are no data on the phytotoxicity of either compound. This lack of data, however, adds no substantial uncertainty to this risk assessment. Diflubenzuron has been extensively tested in both the laboratory and field studies for efficacy in the protection of terrestrial plants from insect pests. If diflubenzuron 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 (Appendix 3a and Appendix 8).

Limited information is available on the toxicity of diflubenzuron and 4-chloroaniline to soil microorganisms. As summarized in Worksheet G02b for diflubenzuron (Supplement 1), exposures of soil microorganisms to diflubenzuron are likely to be below a level of concern for sensitive species by a factor of over 600 at the upper range of plausible exposure – i.e., an HQ of 0.0016. For 4-chloroaniline, the toxicity value for microorganisms is 1000 ppm. As noted above, the highest estimated peak concentration of 4-chloroaniline in soil is 0.0026 ppm (Table 4-3). The resulting HQ is 2.6×10^{-6}, below the level of concern by over 350,000.

4.4.3. Aquatic Organisms

4.4.3.1. Aquatic Vertebrates – As with terrestrial vertebrates, the risk assessment for fish is unequivocal. There is no indication that diflubenzuron or 4-chloroaniline associated with the degradation of diflubenzuron will approach a level of concern.

The highest hazard quotient for diflubenzuron is 0.002 – i.e., longer term exposures to sensitive fish species (Worksheet G03b in Supplement 1). This is below the level of concern by a factor of 500. The toxicity of diflubenzuron has been assayed in relatively few fish species and it is likely that the most sensitive species of fish has not been identified. Nonetheless, there is no basis for asserting that species variability will encompass the factor of 500 associated with the highest HQ for diflubenzuron.

The risk characterization for 4-chloroaniline is virtually identical. The highest hazard quotient is 0.001. Below the level of concern by a factor of 1000 (Worksheet G03, Supplement 2).

4.4.3.2. Aquatic Invertebrates

4.4.3.2.1. Diflubenzuron – As noted by U.S. EPA (1997a), risks to aquatic invertebrates in some applications of diflubenzuron may be substantial – i.e., direct applications to standing bodies of water for mosquito control and forestry uses involving direct applications to bogs, swamps or other standing bodies of water (U.S. EPA 1997a, p. 64). These types of applications, however, are not used in and are thus not relevant to USDA programs for the control of the gypsy moth.

In USDA programs for control of the gypsy moth, risks to aquatic invertebrates appears to be substantially less than risks to terrestrial invertebrates. As noted in Section 2.3, USDA will use a 100 to 500 foot buffer between the application site of diflubenzuron and bodies of open water. While it is possible that small streams could be over-sprayed in aerial applications if the stream is not visible from the air, the covering foliar canopy would intercept some of the diflubenzuron which would in turn reduce the initial concentrations in stream water.

Based on the exposure assessments conducted in this risk assessment, which are consistent with several other exposure assessments as well as a number of relevant monitoring studies (Table 3-7), only the most sensitive species of aquatic invertebrates are likely to be adversely affected based on central estimates of plausible peak exposures. The central estimate of the hazard quotient for sensitive daphnids is only 1.3 (Worksheet G03a, Supplement 1). Typically, hazard quotients are rounded to a single significant digit. Thus, this hazard quotient reaches but does not exceed a level of concern. Based on central estimates of longer term exposures, all hazard quotients are less than 1 (Worksheets G03b, Supplement 1).

At the upper ranges of plausible peak exposures, the level of concern is reached for crabs (HQ=1), modestly exceeded for *Ceriodaphnia* and copepods (HQ=2), and exceeded by a factor of 5 for *Daphnia*. For *Daphnia*, LC_{50} values are only modestly above the NOEC (Table 4-8) and substantial mortality in these species would be plausible. At the upper range of longer term exposures, the hazard quotient exceeds a value of 1 only for *Daphnia* – i.e., HQ=3. This is in the range in which longer term effects on *Daphnia* productivity would be expected and such effects have been observed in field studies (Ali and Mulla 1978b).

Thus, based on the available toxicity data and dose response assessment, the risk characterization for aquatic invertebrates is highly dependant on site-specific conditions. In areas in which water contamination is likely to be minimal – i.e., areas with relatively low rainfall and areas in which drift can be controlled and runoff is limited – it is likely that no or only minimal effects would be observed (e.g., the field study by Ali et al. 1988). During applications in which drift or direct deposition is not controlled well or in areas in which soil losses from runoff and sediment are likely, acute effects on some aquatic invertebrates are plausible and longer term effects on sensitive species could occur.

That any of these effects would result in substantial secondary effects does not seem likely. A large number of field studies are available on diflubenzuron (Appendix 3b) that indicate direct effects on several species of invertebrates at concentrations in water that are above those that would be encountered in many applications for the control of the gypsy moth (see Section 4.1.3.3 for discussion). In addition, the only studies that suggest substantial secondary effects – such as decreased growth in fish – are litoral enclosure studies (Moffett and Tanner 1995; Tanner and Moffett 1995) in which fish were limited in their ability to seek prey. None of the field studies involving free-ranging fish have reported secondary effects other than a change in prey that are consumed.

4.4.3.2.2. 4-Chloroaniline **–** The risks to aquatic invertebrates associated with 4-chloroaniline are insubstantial relative to the risks associated with diflubenzuron. The highest hazard quotient is 0.2, associated with peak exposures to 4-chloroaniline in water.

4.4.3.3. Aquatic Plants and Microorganisms – Risks to aquatic plants and microorganisms appear to be low. There is essentially no identifiable risk associated with diflubenzuron. The highest hazard quotient is 0.04 and is associated with peak exposures to sensitive aquatic plants (Worksheet G03a, Supplement 1). Peak risks associated with 4-chloroaniline are somewhat higher, 0.2, the HQ associated with peak exposures to aquatic plants (Worksheet G03, Supplement 2).

A more plausible risk to aquatic plants may involve secondary effects – increased algal populations – associated with mortality in aquatic grazers such as Cladocerans. This effect has been noted in the mesocosm study by Boyle et al. (1996) . Apperson et al. (1977) noted a decrease in the concentration of a blue-green algae (*Anabaena* species) but no effect on diatoms or green algae. It is unclear if the effect was a primary, secondary, or incidental effect.

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Table 2-1. Selected physical and chemical properties of diflubenzuron [1]

Synonyms and trade names	DFB; Difluron; Dimilin; Duphacid; DU 112307; ENT 29054; Micromite; OMS 1804; PH 60-40; TH-6040
U.S. EPA Reg. No.	400-465 and 400-474 (C&P Press, 2003)
CAS number	35367-38-5 (USDA/ARS 1995)
Molecular weight	310.69 (USDA/ARS 1995; Meylan and Howard 1995)
Molecular formula	$C_{14}H_9ClF_2N_2O_2$ (USDA/ARS 1995; Budavari 1989)
SMILES Notation	O=C(NC(=O)c(c(F)ccc1)c1F)Nc(ccc(c2)Cl)c2
Appearance/state, ambient	Solid (USDA/ARS 1995)
Melting point	230 to 232 °C (USDA/ARS 1995)
Vapor pressure	0.00012 mPa (USDA/ARS 1995)
Water solubility (mg/L)	≃0.3 (Budavari 1989) 0.08 at 25°C (USDA/ARS 1995; Knisel et al. 1992) 0.0888 mg/L in deionized, 0.0926 mg/L in field water (Mabury and Crosby 1996)
log K_{ow}	3.89 (USDA/ARS 1995) [i.e., $K_{ow} = 10^{3.89} = 7762$] 3.59 (estimated) (Meylan and Howard 1995) 3.88 (experimental) (Meylan and Howard 1995) 3.83 ±0.02 (Marsella et al. 2000)
Koc	135.3 (organic soil) (Sundaram et al. 1997) 332.0 (silty clay loam) (Sundaram et al. 1997) 8700 (NOS) (USDA/ARS 1995) 10000 (Knisel and Davis 2000)
Kd	17.59 (organic soil) (Sundaram et al. 1997) 16.42 (silty clay loam) (Sundaram et al. 1997)
Foliar halftimes	9.3 days (Sundaram 1986, 1996) 8 days, 20-80% loss (Wimmer et al. 1993[2]) 29 days (hardwood, van den Berg 1986) 36 days (conifer, van den Berg 1986)
Foliar washoff	50% to 100% depending on formulation, intensity of rainfall, and time of rain after application (Sundaram and Sundaram 1994)
Litter halftimes	8.36 days (Sundaram 1986, 1996)
Soil halftimes	sterile: 346 days in sand and muck (NOS)(Chapman et al. 1985) natural: 18.7 days in sand and muck (NOS)(Chapman et al. 1985) 7.49 days (field study, Sundaram 1986, 1996)
Water photolysis halftime	17±4 hours at pH 7 in distilled water (Marsella et al. 2000) 8±2 hours at pH 9 in distilled water (Marsella et al. 2000) 12.3±0.7 hours at pH 9 in stream water (Marsella et al. 2000)
Aerobic microbial halftime (soil/water)	25.7 days for DFB; 39.7 days for 4-chlorophenylurea (Dzialo and Maynard 1999) 50 hours [2.1 days] (Walstra and Joustra 1990) 5.4 days in water, 8.6 days in sediment (Willard 2000a)
Anaerobic microbial halftime (soil/water)	34 days (Thus et al. 1991)
Water halftime (NOS)	0.97 (0.77-1.16) days without aeration (Anton et al. 1993)
Henry's law constant	0.00047 Pa m^3/mol at 25°C (USDA/ARS 1995) 0.234 ±0.002 Pa×m^3/mole at 20°C (Mabury and Crosby 1996)

[1] Specific environmental fate parameters used in modeling are discussed in Section 3.2.
[2] Reflects initial losses. Remaining DFB much more persistent.

Table 2-2: Commercial formulations of diflubenzuron [1]

Formulation (Supplier)	Type of formulation	%DFB (w/w) [2] (Concentration)	Application Rates [3]		Uses
			Single	Total for year	
Adept (Uniroyal)	Water Soluble Bags	25%	N/A	N/A	Ornamentals
Dimilin 2L (Uniroyal)	Aqueous flowable	22% (2 lbs/gallon)	2-16 fl oz/acre	24 fl oz/acre	Trees and various crops
Dimilin 4L (Uniroyal)	**Liquid**	**40.4 % (4 lbs/gallon)**	**0.5-2 fl oz/acre**	**2 fl oz/acre**	**Forests, ground or aerial.**
Dimilin 25W [4] (Uniroyal)	**Wettable powder**	**25%**	**1-4 oz/acre**	**4 oz/acre**	
Dimilin SC (Uniroyal)	Liquid	40.4 % (4 lbs/gallon)	N/A	N/A	Mushrooms and ornaments
Micromite 25W [5] (Uniroyal)	**Wettable powder**	**25%**	**1-4 oz/acre**	**4 oz/acre**	**Forests, ground or aerial.**
Micromite 25WS (Uniroyal)	Water Soluble Bags	25%	1.25 lbs/acre	3.75 lbs/acre	Citrus crops, ground or aerial
Micromite 25WGS (Uniroyal)	Water Dispersible Granules	80%	6.25 oz/acre	18.75 oz/acre	Citrus crops, ground or aerial

[1] Source: Specimen labels from C&P Press, 2004. Only products in bold font are labeled for gypsy moth.
[2] The remainder of the product formulation is classified as *inerts*. See text for discussion.
[3] All application rates are expressed in amount (lb or oz) of formulation not amounts of active ingredient per acre. N/A indicated that the product is not labeled for broadcast applications. For products labeled for gypsy moth, the range of application rates are those that apply to the gypsy moth.
[4] A separate formulation is available for mushrooms and ornamentals.
[5] The registration for this formulation has been canceled (U.S. EPA/OPP 2002b)

TABLE 2-3: Use of diflubenzuron by USDA from 1995 to 2002 for Suppression, Eradication, and Slow the Spread [1]

Year	Suppression	Eradication	Slow the Spread	Total
1995	161,231			161,231
1996	111,362	6	1,248	112,616
1997	16,447			16,447
1998	757			757
1999	5,275		1,047	6,322
2000	18,090			18,090
2001	187,784		650	188,434
2002	131,601		3,938	135,539
2003	25,124			25,124
Total Acres	657,671	6	6,883	664,560
% of Total	98.96%	0.001%	1.04%	

[1] Source: ***GMDigest***, Morgantown, WV (http://na.fs.fed.us/wv/gmdigest/)

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

Chemical Specific Parameters

Parameter	Clay	Loam	Sand	Comment/ Reference
Halftimes (days)				
Aquatic Sediment	34	34	34	Thus et al. 1991
Foliar	9.3	9.3	9.3	Sundaram 1986, 1996
Soil	10	1.1	2.1	Note 1
Water		5.4		Note 2
Ko/c, mL/g		8700		Note 3
K_d, mL/g	261	130	26.1	Note 4
Water Solubility, mg/L		0.0926		Mabury and Crosby 1996, field sample
Foliar wash-off fraction		0.5		Note 5
Fraction applied to foliage		0.8		
Fraction applied to soil		0.2		

Note 1 Value for sand taken as reported half-time of 50 hours (2.0833 days) taken from Walstra and Joustra 1990. Value for loam taken as reported half-time in silt-loam from Thus and van der Laan-Straathof 1994. No studies on aerobic soil metabolism in clay were found. The value of 10 days is taken from Knisel and Davis (2000) as an upper range.

Note 2 Value for microbial halftime in water from Willard 2000a. Halftimes may be substantially less under conditions where photolysis is the principal route of degradation. See Table 2-1.

Note 3 A very wide range of Koc values (about 135 to 10,000) have been reported (see Table 2-1). The value of 8700 is recommended by USDA/ARS (1995) and is close to the value of 10,000 recommended by Knisel and Davis (2000).

Note 4 Based on the general relationship: Kd = Koc × OC using OC values of 0.003 for sand, 0.015 for loam, and 0.030 for clay (SERA 2003b).

Note 5 This is highly variable. Knisel and Davis (2000) recommend 0.05. The higher value of 0.5 is consistent with the field studies by Sundaram and Sundaram (1994) and Wimmer et al. (1993).

Site Parameters

(see SERA 2004, TD 2004-02.04a dated February 8, 2004 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. 10 hectare square field (1093' by 1093') with a root zone of 12 inches.

Table 3-2: Summary of modeled concentrations of diflubenzuron 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	0.04113	5.17705	0.00000	0.00000	0.00000	0.00000
20	0.56	0.11543	14.59505	0.00000	0.00000	0.00000	0.00000
25	0.69	0.20602	26.22114	0.00000	0.00000	0.00000	0.00000
50	1.39	0.60485	81.46441	0.00000	0.00000	0.00000	0.00002
100	2.78	1.02559	156.23308	0.03588	11.68278	0.00000	0.00028
150	4.17	1.04171	199.48431	0.09107	29.67516	0.00000	0.00105
200	5.56	0.97117	229.82322	0.15544	50.70660	0.00001	0.00258
250	6.94	0.88544	253.52663	0.22002	71.88424	0.00045	0.13780
Application rate:		**0.0624**	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	0.003	0.32305	0	0	0	0
20	0.56	0.007	0.91073	0	0	0	0
25	0.69	0.0129	1.6362	0	0	0	0
50	1.39	0.0377	5.08338	0	0	0	0
100	2.78	0.064	9.74894	0.002	0.72901	0	0
150	4.17	0.065	12.4478	0.006	1.85173	0	0
200	5.56	0.0606	14.341	0.01	3.16409	0	0
250	6.94	0.0553	15.8201	0.0137	4.48558	0	0.009

[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 diflubenzuron 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	0.00704	0.07849	0.00000	0.00000	0.00000	0.00000
20	0.56	0.01700	0.26465	0.00000	0.00000	0.00000	0.00000
25	0.69	0.02989	0.56583	0.00000	0.00000	0.00000	0.00000
50	1.39	0.11171	3.32693	0.00000	0.00000	0.00000	0.00000
100	2.78	0.29257	12.37300	0.01577	1.63558	0.00000	0.00007
150	4.17	0.39616	23.59907	0.04933	5.81660	0.00000	0.00033
200	5.56	0.45379	35.86106	0.09695	12.41986	0.00001	0.00096
250	6.94	0.48619	48.35946	0.15210	20.70574	0.00035	0.05865
Application rate:		**0.0624**	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	0.0004	0.0049	0	0	0	0
20	0.56	0.00106	0.016514	0	0	0	0
25	0.69	0.00187	0.035308	0	0	0	0
50	1.39	0.00697	0.2076004	0	0	0	0
100	2.78	0.018256	0.7720752	0.001	0.1020602	0	0
150	4.17	0.02472	1.472582	0.00308	0.3629558	0	0
200	5.56	0.028317	2.2377301	0.00605	0.7749993	0	0
250	6.94	0.030338	3.0176303	0.00949	1.2920382	0	0.00366

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

Table 3-4: Chemical and site parameters used in GLEAMS modeling for 4-chloroaniline.

Chemical Specific Parameters

Parameter	Clay	Loam	Sand	Comment/ Reference
Halftimes (days)				
Aquatic Sediment		150		Note 2
Foliar		0.16		Note 2
Soil		37.5		Note1
Water		151		Note 2
Ko/c, mL/g		72		Note 1
K_d, mL/g	2.2	1.1	0.22	Note 3
Water Solubility, mg/L		3900		Note 1
Foliar wash-off fraction		0.5		
Coefficient of transformation		0.41		Note 4

Note 1 Estimated from EPI-Suite (Meylan and Howard 1998, 2000)

Note 2 WHO 2003. Foliar halftime is not given explicitly in WHO (2003) and is estimated here based on the atmospheric halftime of 3.9 hours.

Note 3 Based on Kd = Ko/c × OC, where OC is the proportion of organic carbon. The OC in sand, loam, and clay is taken as 0.003 for sand, 0.015 for loam, and 0.030 for clay (SERA 2004).

Note 4 This is the ratio of the molecular weight of chloroaniline (127.57) to that of diflubenzuron (310.69). See discussion by Knisel and Davis (2000, p. 110).

Table 3-5: Summary of modeled concentrations of 4-chloroaniline 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	0.06559	4.19361	0.00048	0.01145	0.11234	2.57651
20	0.56	0.15452	10.45786	0.01616	0.32734	0.36403	10.48046
25	0.69	0.22436	15.84683	0.03969	0.85101	0.55917	19.16073
50	1.39	0.31156	27.90970	0.16080	4.59647	0.77622	44.23856
100	2.78	0.29226	30.80407	0.22906	9.17859	0.59128	52.72812
150	4.17	0.13293	24.52481	0.20128	9.67567	0.45074	51.02312
200	5.56	0.06009	14.09093	0.16267	8.73307	0.36145	49.79360
250	6.94	0.01924	5.74944	0.12680	7.21420	0.30139	47.06395
	Application rate:	**0.0624**	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	0.00409	0.2616813	0	0.0007	0.00701	0.1607742
20	0.56	0.00964	0.6525705	0.00101	0.020426	0.022715	0.6539807
25	0.69	0.014	0.9888422	0.00248	0.053103	0.034892	1.1956296
50	1.39	0.019441	1.7415653	0.010034	0.2868197	0.048436	2.7604861
100	2.78	0.018237	1.922174	0.014293	0.572744	0.036896	3.2902347
150	4.17	0.00829	1.5303481	0.01256	0.6037618	0.028126	3.1838427
200	5.56	0.00375	0.879274	0.010151	0.5449436	0.022554	3.1071206
250	6.94	0.0012	0.3587651	0.00791	0.4501661	0.018807	2.9367905

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

Table 3-6: Summary of modeled concentrations of 4-chloroaniline 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	0.31929	0.69851	0.00288	0.00477	0.65741	1.11311
20	0.56	0.56688	1.80242	0.07465	0.15746	1.72523	4.20894
25	0.69	0.74573	2.90175	0.16734	0.40004	2.48876	7.61750
50	1.39	1.04158	6.43073	0.63473	2.28508	3.46266	18.05225
100	2.78	1.01591	8.41740	0.97319	5.00787	2.89735	23.03849
150	4.17	0.60259	6.77759	0.90309	5.52346	2.34727	22.92303
200	5.56	0.29679	4.08394	0.75792	5.16526	1.96069	22.29465
250	6.94	0.10055	1.77278	0.60774	4.47424	1.68309	21.01092
Application rate:		**0.0624**	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	0.019924	0.043587	0.0002	0.0003	0.041022	0.069458
20	0.56	0.035373	0.112471	0.00466	0.00983	0.1076544	0.2626379
25	0.69	0.046534	0.1810692	0.010442	0.024963	0.1552986	0.475332
50	1.39	0.064995	0.4012776	0.039607	0.142589	0.21607	1.1264604
100	2.78	0.063393	0.5252458	0.060727	0.3124911	0.1807946	1.4376018
150	4.17	0.037602	0.4229216	0.056353	0.3446639	0.1464696	1.4303971
200	5.56	0.01852	0.2548379	0.047294	0.3223122	0.1223471	1.3911862
250	6.94	0.00627	0.1106215	0.037923	0.2791926	0.1050248	1.3110814

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

Table 3-7: Estimated Environmental Concentrations (µg/L or ppb) of diflubenzuron in ponds and streams.

Scenario	Peak	Long-Term Average
MODELING FOR THIS RISK ASSESSMENT (0.0624 lb/acre or 70 g/ha)		
Stream		
Direct Spray [1]	5.7	N/A
100 Foot buffer [1]	0.11	N/A
GLEAMS (Table 3-2)	2 (<0.01 to 16)	0.01 (0 to 0.06)
Pond		
Direct Spray [2]	3.5	N/A
100 Foot buffer [2]	0.07	N/A
GLEAMS (Table 3-3)	0.2 (<0.005 to 3) at 0.06 lb/ac	0.007 (0 to 0.03) at 0.06 lb/ac
OTHER MODELING		
USDA (1995)	16.01 (stream, direct spray) 2.76 to 13.14 (stream, runoff) 1.22 (pond)	N/A
U.S. EPA/OPP 1997a. Pond: citrus crops	3.4 ppb at 6x 0.06 lb/ac 8.1 ppb at 0.67 lb/ac	0.74 ppb at 6x 0.06 lb/ac 0.87 ppb at 0.67 lb/ac
U.S. EPA/OPP 1997a. Pond: direct applications to water in forestry	11.7 ppb at 0.05 lb/ac 22.8 ppb at 0.07 lb/ac 46.2 ppb at 0.15 lb/ac 91.8 ppb at 0.32 lb/ac	N/A
Harned and Relyea 1997	Peak concentration of 1 ppb at an application rate of 350 g/ha. Longer term concentration of about 0.1 ppb. See text for discussion.	
Schocken et al. 2001	Peak concentrations of about 0.2 to 0.3 ppb in ponds and 0.9 ppb in steams at an application rate of 0.125 lb/acre. See text for discussion.	

[1] See Worksheet 10b
[2] See Worksheet 10a

Table 3-8: Concentrations of diflubenzuron in surface water used in this risk assessment (see Section 3.2.3.4.6 for discussion).

At application rate:	**0.0624**	**lb/acre**	
		Peak Concentration (ppb or µg/L)	Longer Term Concentration (ppb or µg/L)
	Central	0.4	0.02
	Lower	0.01	0.001
	Upper	16	0.1
Water contamination rate [1]		**mg/L per lb/acre applied.**	
		Peak Concentration (mg/L per lb/acre)	Longer Term Concentration (mg/L per lb/acre)
	Central	6.41e-03	3.21e-04
	Lower	1.60e-04	1.60e-05
	Upper	2.56e-01	1.60e-03

[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 A04 for diflubenzuron. This rate is adjusted to the program application rate in all worksheets involving exposure to contaminated water.

Table 3-9: Concentrations of 4-chloroaniline in surface water used in this risk assessment (see Section 3.2.3.4.7 for discussion).

At application rate:	**0.0624**	**lb/acre**	
		Peak Concentration (ppb or µg/L)	Longer Term Concentration (ppb or µg/L)
	Central	0.5	0.05
	Lower	0.00003	0.0002
	Upper	3	0.2
Water contamination rate [1]		**mg/L per lb/acre applied.**	
		Peak Concentration (mg/L per lb/acre)	Longer Term Concentration (mg/L per lb/acre)
	Central	8.01e-03	8.01e-04
	Lower	4.81e-07	3.21e-06
	Upper	4.81e-02	3.21e-03

[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 A04 for 4-chloroaniline. This rate is adjusted to the program application rate in all worksheets involving exposure to contaminated water.

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

Range of Application Rates (g/ha)	Species	
	No Adverse Effects	**Adverse Effects**
<20	ants (Catangui et al. 1996) *Cotesia melanoscelus* (GM parasitic wasp) (Webb et al. 1989)	grasshoppers (Jech et al. 1993)
20 - <40	lacewing and beetles (Ables et al. 1977) carabids, crickets, lice (Butler et al. 1997) honey bee (Matthenius1975) honey bee [×8](Robinson 1978,1979)	gypsy moth and macrolepidoptera (Butler et al. 1997) grasshopper (Everts 1990) *Apanteles melanoscelus* # (GM parasitic wasp) (Madrid and Stewart1981)
40 - < 60	lacewing and beetles (Ables et al. 1977)	
60 - < 100	*Ooencyrtus kuvanae* (GM parasitic wasp) (Brown and Respicio 1981) lacewing and beetles (Deakle and Bradley1982) honey bee (Matthenius1975) sucking herbivorous insects, microlepidoptera, and predaceous arthropods(Martinat et al. 1988) spiders* and orthopteroid*(Martinat et al. 1993) mites and springtails (Perry et al. 1997) spiders** (Perry et al. 1997) non-lepidopteran insects (Sample et al. 1993a,b) mites* and collembolans* (Van Den Berg 1986)	grasshopper (Everts 1990) grasshoppers, moths, carabid beetles (Butler 1993) lepidoptera (Sample et al. 1993a,b) macrolepidoptera and other herbivorous insects (Martinat et al. 1988) Yellow jacket wasp (Barrows et al. 1994)
100 - < 150	ants (Weiland 2000) *Psylla* parasites and predators (Westigard 1979) lacewing and beetles (Ables et al. 1977) honey bee (Emmett and Archer 1980) honey bee [×8](Robinson 1978,1979)	soil mites (Blumberg 1986) Yellow jacket wasp (Weiland 2000)
150 - < 200	various arthropod predators (Keever et al. 1977)	lepidopteran egg mortality (low) (Kumar et al. 1994) mites (Marshall 1979)
200 - < 300	ants (Weiland 2000) carabid beetles (Heinrichs et al. 1979)	lacewing and beetles (Ables et al. 1977) mites (Marshall 1979) borer weevil (Schroeder 1996) predatory damsel bugs and sucking insects (Turnipseed et al. 1974) Yellow jacket wasp (Weiland 2000) *Psylla* parasites and predators (Westigard 1979) flying insects, esp. midges, gnats, and mosquitoes (Wilson and Wan 1977a)
≥ 300	honey bee (Buckner et al. 1975) honey bee (Emmett and Archer 1980) honey bee and other beneficial insects (Schroeder 1980)	lepidopteran egg mortality (high) (Kumar et al. 1994) *Psylla* parasites and predators (Westigard 1979)

[1] Studies summarized in Appendix 3a. See text for discussion. A single asterisk (*) indicates transient or equivocal effects. A double asterisk (**) indicates effects that were secondary to decrease in prey. The # symbol indicates an effect clearly due to toxicity. GM used as abbreviation for gypsy moth. Multiple applications are indicated in brackets with a × symbol followed by the number of applications.

Table 4-2: Summary of modeled concentrations of diflubenzuron in soil (all units are mg/kg or ppm)

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	0.00841	0.14004	0.00092	0.11651	0.00169	0.12485
10	0.28	0.00926	0.14004	0.00106	0.11652	0.00194	0.12484
15	0.42	0.00924	0.13992	0.00106	0.11653	0.00193	0.12484
20	0.56	0.00918	0.13962	0.00106	0.11653	0.00193	0.12484
25	0.69	0.00910	0.13914	0.00106	0.11653	0.00193	0.12484
50	1.39	0.00834	0.13431	0.00106	0.11653	0.00192	0.12484
100	2.78	0.00650	0.11909	0.00104	0.11450	0.00190	0.12484
150	4.17	0.00412	0.09305	0.00099	0.10879	0.00188	0.12484
200	5.56	0.00234	0.06298	0.00091	0.09889	0.00186	0.12484
250	6.94	0.00104	0.05236	0.00080	0.08527	0.00184	0.12478
	Application rate:	**0.0624**	lbs/acre				
			Concentration at above application rate				
5	0.14	5.2e-04	0.00874	5.7e-05	0.00727	1.1e-04	0.00779
10	0.28	5.8e-04	0.00874	6.6e-05	0.00727	1.2e-04	0.00779
15	0.42	5.8e-04	0.00873	6.6e-05	0.00727	1.2e-04	0.00779
20	0.56	5.7e-04	0.00871	6.6e-05	0.00727	1.2e-04	0.00779
25	0.69	5.7e-04	0.00868	6.6e-05	0.00727	1.2e-04	0.00779
50	1.39	5.2e-04	0.00838	6.6e-05	0.00727	1.2e-04	0.00779
100	2.78	4.1e-04	0.00743	6.5e-05	0.00714	1.2e-04	0.00779
150	4.17	2.6e-04	0.00581	6.2e-05	0.00679	1.2e-04	0.00779
200	5.56	1.5e-04	0.00393	5.7e-05	0.00617	1.2e-04	0.00779
250	6.94	6.5e-05	0.00327	5.0e-05	0.00532	1.1e-04	0.00779

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

Table 4-3: Summary of modeled concentrations of 4-chloroaniline in soil (all units are mg/kg or ppm)

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	0.00672	0.02893	0.00680	0.04917	0.00750	0.04216
10	0.28	0.00655	0.02685	0.00626	0.04550	0.00666	0.04159
15	0.42	0.00699	0.02697	0.00709	0.04556	0.00751	0.04167
20	0.56	0.00691	0.02665	0.00734	0.04562	0.00728	0.04168
25	0.69	0.00668	0.02618	0.00748	0.04566	0.00685	0.04157
50	1.39	0.00360	0.02252	0.00737	0.04582	0.00493	0.04032
100	2.78	0.00631	0.01739	0.00622	0.04519	0.00323	0.04015
150	4.17	0.00307	0.01146	0.00529	0.04326	0.00254	0.04001
200	5.56	0.00142	0.00759	0.00450	0.03994	0.00216	0.03997
250	6.94	0.00050	0.00357	0.00375	0.03540	0.00193	0.03999
Application rate:		**0.0624**	lbs/acre				
		Concentration at above application rate					
5	0.14	4.2e-04	0.00181	4.2e-04	0.00307	4.7e-04	0.00263
10	0.28	4.1e-04	0.00168	3.9e-04	0.00284	4.2e-04	0.0026
15	0.42	4.4e-04	0.00168	4.4e-04	0.00284	4.7e-04	0.0026
20	0.56	4.3e-04	0.00166	4.6e-04	0.00285	4.5e-04	0.0026
25	0.69	4.2e-04	0.00163	4.7e-04	0.00285	4.3e-04	0.00259
50	1.39	2.2e-04	0.00141	4.6e-04	0.00286	3.1e-04	0.00252
100	2.78	3.9e-04	0.00109	3.9e-04	0.00282	2.0e-04	0.00251
150	4.17	1.9e-04	0.0007	3.3e-04	0.0027	1.6e-04	0.0025
200	5.56	8.9e-05	0.0005	2.8e-04	0.00249	1.3e-04	0.00249
250	6.94	3.1e-05	0.0002	2.3e-04	0.00221	1.2e-04	0.0025

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

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

Organism	Endpoint	Toxicity Value	Reference, Species
Mammals	Acute NOAEL	1118 mg/kg	Blaszcak 1997a, rats [Dimilin 2L]
	Chronic NOAEL	2 mg/kg/day	Greenough et al. 1985, dogs
Birds	Acute NOAEL	2500 mg/kg	Alsager and Cook 1975, blackbirds
	Chronic NOAEL	110 mg/kg	Beavers et al. 1990b, quail
Terrestrial arthropods		***See Table 4-5 for toxicity values***	
Soil invertebrates			
Earthworm	NOEC	780 mg/kg soil	Berends et al. 1992
Soil microorganisms			
Sensitive	50 ppm LOEC	50 ppm ÷ 10	Townshend et al. 1983
Tolerant	100 pp NOEC	100 ppm	Townshend et al. 1983
Fish Acute			
Sensitive	LC_{50}	25 mg/L	Johnson and Finley 1980, yellow perch
Tolerant	LC_{50}	500 mg/L	Reiner and Parke 1975, fathead minnow
Fish Chronic			
Sensitive	Reproductive NOEC	0.05 mg/L	Livingston and Koenig 1977, mummichog
Tolerant	Reproductive NOEC	0.1 mg/L	Cannon and Krize 1976, fathead minnow
Aquatic Invertebrates		***See Table 4-6 for toxicity values***	
Aquatic Plants			
Sensitive	NOEC for growth	0.045 mg/L	Hansen and Garton 1982a, *Selenastrum capricornutum*
Tolerant	NOEC for growth	0.38 mg/L	Thompson and Swigert 1993c, *Navicula pelliculosa*
Aquatic Microorganisms	NOEC for respiration	0.05 mg/L	Kreutzweiser et al. 2001 [4.3.3.4]

[1] NOECs are used directly when available. When only a LOEC is available, the LOEC is divided by 10 to approximate the NOEC. This is indicated by the "÷10" following the LOEC.

Table 4-5: Diflubenzuron toxicity values used in risk assessment for terrestrial arthropods (see Table 4-1 for additional details).

Organism	Endpoint	Toxicity Value[1]	Reference
Grasshoppers	Field LOAEL	22 g/ha ÷ 10	Jech et al. 1993
Apanteles melanoscelus[2]	Field LOAEL	30 g/ha ÷ 10	Madrid and Stewart1981
Macrolepidoptera	Field LOAEL	35 g/ha ÷ 10	Butler et al. 1997
Mandibulate herb. insects	Field LOAEL	70 g/ha ÷ 10	Martinat et al. 1988
Ooencyrtus kuvanae[2]	Field NOAEL	67 g/ha	Brown and Respicio 1981
Microlepidoptera	Field NOAEL	70 g/ha	Martinat et al. 1988
Predaceous arthropods	Field NOAEL	70 g/ha	Martinat et al. 1988
Sucking herbaceous insects	Field NOAEL/LOAEL	70/281 g/ha	Martinat et al. 1988/Turnipseed et al. 1974
Spiders	Field NOAEL	70 g/ha	Martinat et al. 1993
Mites and collembolans	Field NOAEL/LOAEL	70/140 g/ha	Perry et al. 1997/Blumberg 1986
ants	Field NOAEL	280	Weiland 2000
Lacewing	Field NOAEL/LOAEL	140/280 g/ha	Ables et al. 1977
Honey bee	Field NOAEL	400 g/ha	Emmett and Archer 1980

[1] Field NOAELs are used directly when available. When only a LOAEL is available, the LOAEL is divided by 10 to approximate the NOAEL. This is indicated by the "÷10" following the LOAEL.
[2] A parasitic wasp to the gypsy moth.

Table 4-6: Diflubenzuron toxicity values used in risk assessment for aquatic invertebrates.

Organism	Endpoint	Toxicity Value ppb or µg/L[1]	Reference
ACUTE (see Table 4-8 for additional details)			
Daphnia	NOEC	0.3	Corry et al. 1995
Ceriodaphnia	NOEC	0.75	Hall 1986
Copepods	NOEC	0.93	Savitz et al. 1994
crabs	NOEC	2	Cunningham and Meyers 1987
rotifers	NOEC	20	Corry et al. 1995
large insects	NOEC	2000	Lahr et al. 2001
molluscs	NOEC	125000	Wilcox and Coffey 1978
LONGER TERM (see Table 4-9 for additional details)			
Daphnia	NOEC	0.04	Surprenant 1988
stoneflies and mayflies	NOEC	0.1	Hansen and Garton 1982b
Ceriodaphnia	NOEC	0.25	Hall 1986
dragonflies	NOEC	0.7	O'Halloran and Liber 1995
ostracods	NOEC	2.5	Liber and O'Halloran 1995
coleoptera and oligochaetes	NOEC	50	Hansen and Garton 1982a
molluscs	NOEC	320	Surprenant 1989

[1] In worksheets, all concentrations in ppb are divided by 1000 to convert to concentrations in ppm or mg/L.

Table 4-7: Summary of 4-chloroaniline toxicity values used in ecological risk assessment

Organism	Duration/Endpoint	Toxicity Value	Reference, species
Mammals	Acute/Toxicity NOAEL	8 mg/kg/day	Used in HHRA
	Chronic/Toxicity NOAEL	1.25 mg/kg/day	Estimated from LOAEL of 12.5 mg/kg/day
Birds	Acute/Toxicity NOAEL	8 mg/kg/day	No data. Uses value for mammals
	Chronic/Toxicity NOAEL	1.25 mg/kg/day	No data. Uses value for mammals
Earthworms	NOEC	540 mg/kg soil	WHO 2003
Soil Microorganisms	NOEC	1000 ppm	Welp and Brummer 1999
Fish			
Acute	LC_{50}	2.4 mg/L	WHO 2003, Bluegill
Chronic	NOEC, reproduction	0.2 mg/L	Bresch et al. 1990, Zebra fish
Aquatic Invertebrates			
Acute	NOEC, mortality	0.013 mg/L	Kuhn et al 1989a
Chronic	NOEC, reproduction	0.01 mg/L	Kuhn et al 1989a
Aquatic plants	EC_{10}	0.02 mg/L	Schmidt and Schnabl 1988, green algae
Aquatic Microorganisms	NOEC (30 min)	5.1 mg/L	Ribo and Kaiser 1984, photobacteria

Table 4-8: Acute toxicity of diflubenzuron in aquatic invertebrates

Concentrations (µg/L or ppb)	No Effect Species/group [conc. ppb](Reference)	Adverse Effect Species/group [conc. ppb](Reference)
0.1 to <1	mysid shrimp[0.12] (Breteler 1987) *Daphnia* [0.3](Corry et al. 1995) *Daphnia* [0.45](Kuijpers 1988) *Ceriodaphnia* [0.75](Hall 1986) copepods [0.93](Savitz et al. 1994)	Mosquito [0.5] (Miura and Takahashi 1974) *Daphnia* [0.7](Corry et al. 1995) *Daphnia* [0.7](Kuijpers 1988) *Daphnia* [0.75, neonate](Majori et al. 1984) fairy shrimp [0.74] (Lahr et al. 2001)
1 to <10	fiddler crabs [2] (Cunningham and Meyers 1987) Horseshoe crabs[4] [5] (Weis and Ma 1987) amphipods [7] (Corry et al. 1995)	gammarids[1](Hansen and Garton 1982a) *Ceriodaphnia* [1.7](Hall 1986) copepods [1.7](Savitz et al. 1994) midges[1.8](Hansen and Garton 1982a) blue crab eggs [1.8] (Lee and Oshima 1998) grass shrimp [3.4](Tourat and Rao 1987) grass shrimp [2-3](Wilson and Costlow 1986) mysid shrimp[2.1]Nimmo et al. 1979
10 to <100	rotifers[20] (Corry et al. 1995) snails [45](Hansen and Garton 1982a)	Mayfly [10] (Miura and Takahashi 1974) Amphipods [13](Corry et al. 1995) *Daphnia* [23, adult](Majori et al. 1984) Dragonfly [50] (Miura and Takahashi 1974) Horseshoe crabs [50] (Weis and Ma 1987)
100 to <1000		beetles [100] (Miura and Takahashi 1974) fiddler crabs [200] (Cunningham and Meyers 1987) tricoptera [250] (Bradt and Williams 1990) grass shrimp[640] (Bionomics-EG&G 1975)
>1000	backswimmer[2] [2000] (Lahr et al. 2001) snail [125,000](Wilcox and Coffey 1978)	midge [560] (Julin and Sanders 1978)

[1] Macrocosm study
[2] No molting during short term exposures
[3] Litoral enclosures
[4] Marginal signs of toxicity

Table 4-9: Chronic toxicity of diflubenzuron in aquatic invertebrates

Concentrations (µg/L or ppb)	No Effect Species/group [conc. ppb](Reference)	Adverse Effect Species/group [conc. ppb](Reference)
>0.01 to 0.1	*Daphnia*[0.04]Surprenant 1988 stream inverts[1] [0.1](Hansen and Garton 1982a[1]) stoneflies and mayflies[0.1] (Hansen and Garton 1982b[1])	*Daphnia* [0.06] U.S. EPA 1997a [5] mysid shrimp[0.075]Nimmo et al. 1979 *Daphnia* [0.09]LeBlanc (1975) *Daphnia*[0.093]Surprenant 1988
>0.1 to 1	*Ceriodaphnia* [0.25](Hall 1986) mayflies, damselflies, and dragonflies[0.7] (O'Halloran and Liber 1995) mixed insects[3] [1](Liber 1995)	*Ceriodaphnia* [0.5](Hall 1986) clodacera and copopods[3][0.7] (Liber and O'Halloran 1995) copepods [0.7-0.9](Wright et al. 1996) grass shrimp (Bionomics-EG&G 1975) grass shrimp [0.7](Tourat and Rao 1987) stream inverts [1] (Hansen and Garton 1982a) stoneflies and mayflies[1] (Hansen and Garton 1982b[1])
>1 to 10	Ostracoda[3][2.5](Liber and O'Halloran 1995)	dipterans[10] (Hansen and Garton 1982a[1]) mixed insects[3] [1.9](Liber 1995) Ostracoda[3][7](Liber and O'Halloran 1995) mayflies, damselflies, and dragonflies[2.5] (O'Halloran and Liber 1995)
>10 to 100	coleoptera, oligochaetes, and gastropods[1][50] (Hansen and Garton 1982a) rotifers[3][30](Liber and O'Halloran 1995)	
>100 to 1000	clams [320](Surprenant 1989)	

[1] Macrocosm study
[2] No molting during short term exposures
[3] Litoral enclosures
[4] Marginal signs of toxicity
[5] Cited in U.S. EPA (1997a) as Beltsville Lab Test 2424. This study is not identified by MRID number or otherwise described.

Table 4-10: Summary of field studies on the effects of diflubenzuron on aquatic invertebrates [1]

Range of Application Rates (g/ha)	Species [conc ppb](Reference)		
	No Adverse Effects	**Adverse Effects with Observed Recovery**	**Adverse Effects with No Observed Recovery**
>0.1 to 1	pond invertebrates [0.2] Ali et al. 1988		
>1 to 10	shrimp, cyclops, and some cladocera (*Bosmina*), worms [3.7] (Ali and Mulla 1978a) worms [7.4] (Ali and Mulla 1978a)	zooplankton mortality and insect emergence [1.8](Wan and Wilson 1977) daphnids and copepods [3.7] (Ali and Mulla 1978a) copepods, shrimp [7.4] (Ali and Mulla 1978a) cladocera, copepods [2.5 to 10](Apperson et al. 1977) cladocerans, copepods and rotifers[10](Boyle et al. 1996)	amphipods [3.7] (Ali and Mulla 1978a) amphipods, daphnids [7.4] (Ali and Mulla 1978a)
>10 to 100	rotifers [13](Colwell and Schaefer 1980)	cladocera [10.4](Lahr et al. 2000) cladocera incl. *Bosmina*, copepods, [13](Colwell and Schaefer 1980)	shrimp [10.4](Lahr et al. 2000)

[1] The concentrations given in braces [] represent peak or typical concentrations shortly after exposure. In all cases, post-application concentrations will decline. See text for discussion.

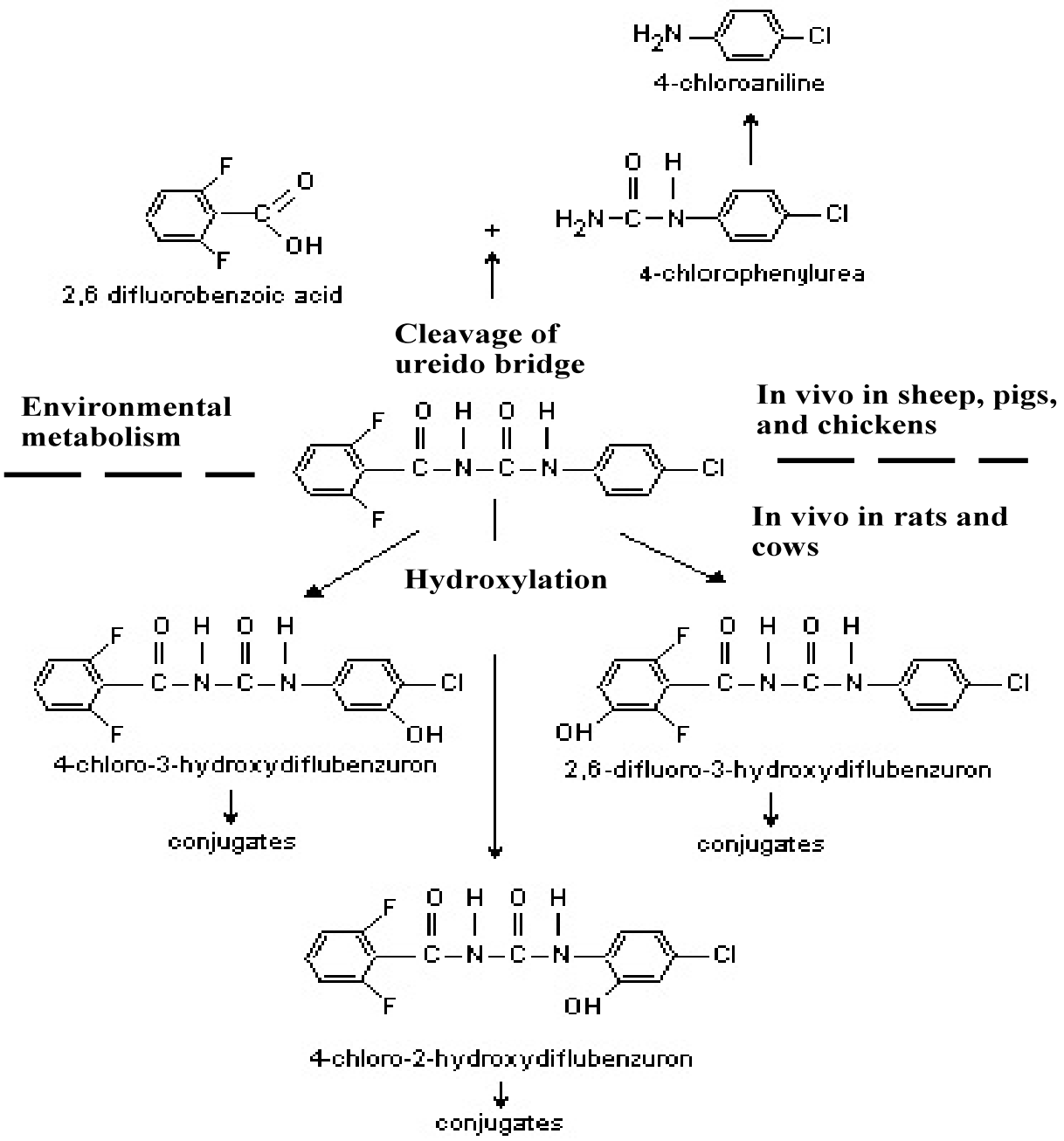

Figure 3-1: Overview of the *In vivo* and environmental metabolism of diflubenzuron (adapted from WHO 1996).

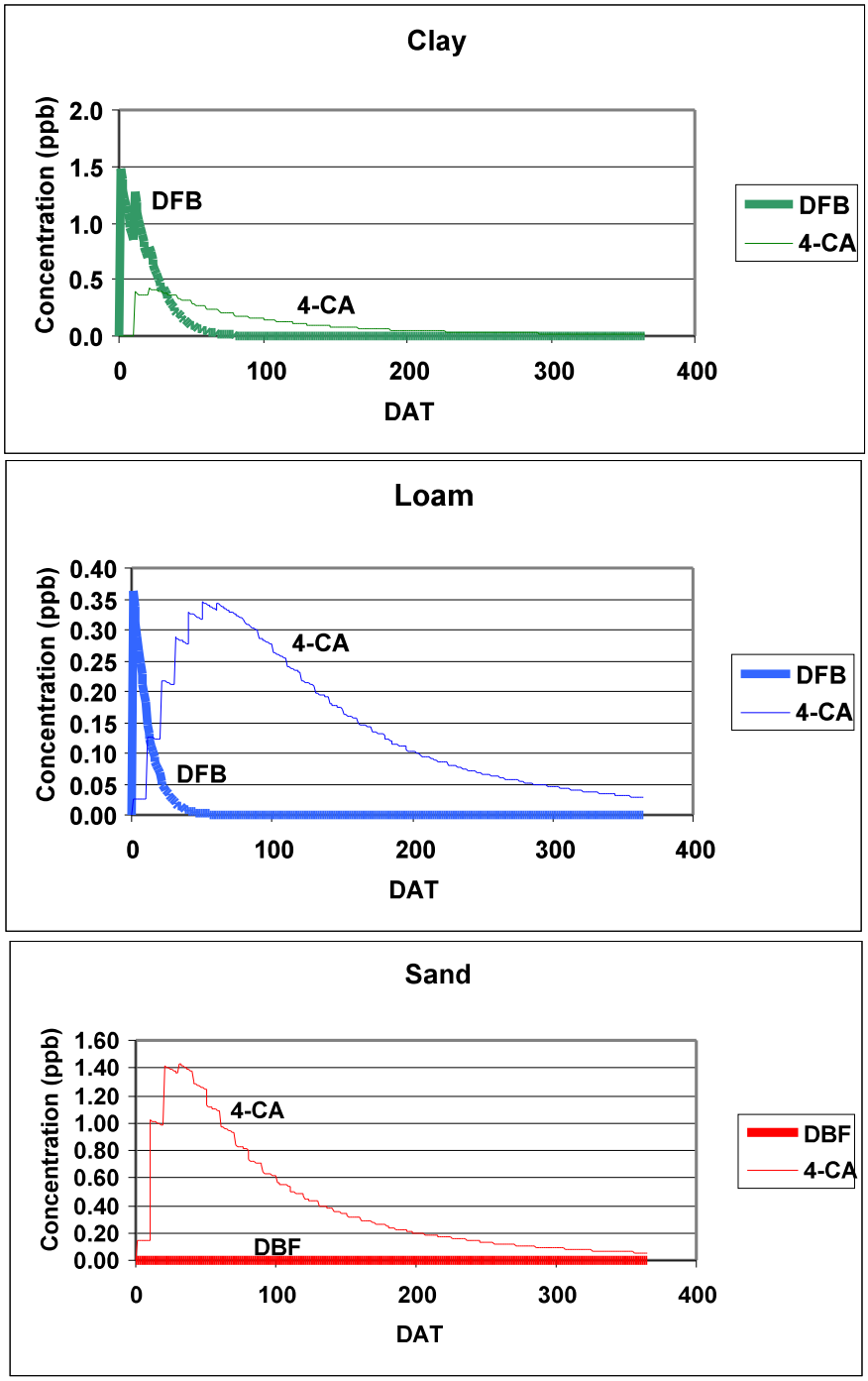

Figure 3-2: Modeled concentrations of diflubenzuron (thick lines) and 4-chloroaniline (thin lines) in ponds at an annual rainfall rate of 150 inches (see text for discussion).

APPENDICES

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Oral			
		Diflubenzuron	
Acute Oral			
Mouse and rat	LD_{50}, technical grade	> 4640 mg/kg	WHO 1996
Mouse and rat	LD_{50},90% concentrate	> 5000 mg/kg	WHO 1996; U.S. EPA 1997a
Mouse and rat	LD_{50}, Du 112307 W.P. 25% (Dimilin WP 25%)	> 40,000 mg Dimilim/kg > 10,000 mg DFB/kg A marginal effects on methemoglobin levels.	Koopman 1977 MRID 00070025
Rat, Sprague-Dawley, 5 males(290-330 g) and 5 females (215-233 g), 9- to 12-weeks old	single gavage dose of 5000 mg/kg Dimilin 2L (22.36% pure)	No mortality. Except for moist rales in two treated rats on the day of dosing, no clinical signs of toxicity, all rats gained weight both 7 and 14 days after dosing, and no abnormalities observed during macroscopic postmortem evaluation. NOEC = 5000 mg/kg as Dimilin 2L 1118 mg/kg as DFB	Blaszcak 1997a MRID 44574504
Subchronic Oral			
Cat (NOS)	0, 30, 100, 300, or 1000 mg/kg/day diflubenzuron for 3 weeks	NOEC (Hb) >1250 mg/kg/day NOEC (%PCV) not estimated NOEC (RBC) not estimated NOEC (reticulocyte count) not estimated NOEC (MetHb) = 30 mg/kg/day NOEC (SulpHb) = 3 mg/kg/day (calculated with regression analysis) NOEC (spleen weight) >1000 mg/kg/day	Keet et al. 1982

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Dogs, beagle, pure-bred, 15 males and 15 females	dietary levels of 10, 20, 40 or 60 ppm (**actual dosages levels of 0.42, 0.84, 1.64, or 6.24 mg/kg/day**) Du 112307 for 13 weeks	No mortality; no clinical signs of toxicity, no adverse effects on food or water consumption, no ocular effects, no treatment-related macroscopic post mortem findings, no adverse effects on organ weights, and no morphological abnormalities considered to be treatment related. At 2 weeks, all laboratory tests were within normal limits; at 4 and 6 weeks, SAP and SGPT were increased among some dogs at 40 or 160 ppm; after 6 weeks, the presence of **methaemoglobin** and other abnormal haemoglobin pigments was apparent in dogs at 160 ppm; after 12 weeks, one dog at 160 ppm had an elevated SGPT level and one dog at 160 ppm and one dog had a greater **methaemoglobin** value than all the other dogs. NOEC = 20 ppm	Chesterman et al. 1974 MRID 00038706
Dog (NOS)	0, 2, 10, 50, or 250 mg/kg/day diflubenzuron for 13 weeks	NOEC (Hb) = 10 mg/kg/day NOEC (%PCV) not estimated NOEC (RBC) >250 mg/kg/day NOEC (reticulocytes) = 50 mg/kg/day NOEC (MetHb) = 50 mg/kg/day NOEC (SulpHb) = 10 mg/kg/day NOEC (spleen weight) not estimated	Keet et al. 1982

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Mice, 40/sex/dose group	in diet concentrations of 0, 80, 400, 2000, 10,000, or 50,000 ppm 97.2% pure, technical grade, air-milled diflubenzuron for 14 weeks with 7-week interim sacrifice. The **calculated mean intake** of diflubenzuron was 9.7, 50.7, 240, 1174, or 6114 mg/kg/day (males) and 11.1, 54.9, 288, 1393, or 7506 mg/kg/day (females) [*cf* page 27]	No treatment-related mortality throughout the study; no significant, treatment-related changes in food consumption or body weight; numerous hematological effects, including statistically significant increases (see pg 29) in Met Hb% and Sulph Hb% in males and females at 400-50,000 ppm; statistically significant increase in spleen weight in males and females at 400-50,000 ppm; statistically significant increase in liver weight of males and females at 2000-50,000 ppm;	Colley et al. 1981 MRID 00114330
Rats, Swiss-albino, males, weighing 90 g, 5/dose group	gavage doses of 96.7 mg/kg of Dimilin in corn oil solution each day for 48 days (i.e., total of 4640 mg/kg of Dimilin)	Mean hemoglobin concentration (g/100 mL blood) was significantly lower than that of controls; mean hematocrit percent of the Dimilin was significantly higher than that of controls.	Berberian and Enan 1989
Rats, Sprague-Dawley, 40/sex/dose group	in diet concentrations of 160, 400, 2000, 10,000, or 50,000 ppm technical grade diflubenzuron for 90 days	No mortality; no clinical signs of toxicity, no adverse effects on body weight or food consumption. Treatment-related adverse effects included a significant increase in **methemoglobin** at weeks 7 and 13 in males at 400, 2000, 10,000, and 50,000 ppm and in females at all dose levels, as well as significant increases in sulfhemoglobin at week 7 in 50,000 ppm males and 10,000 and 50,000 ppm females, and at week 13 in males at 10,000 and 50,000 ppm and in females at 2000, 10,000, and 50,000 ppm. Other pathological, treatment-related changes included decreases in hematocrit and hemoglobin values and the erythrocyte count and an increase in the number of reticulocytes, increases in absolute liver weight and absolute and relative spleen weights, and enlargement of the spleen. NOEC (for males only) = 160 ppm	Burdock et al. 1980 MRID 00064550

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Sheep (NOS)	0, 25, 125, or 500 mg/kg/day diflubenzuron for 13 weeks.	NOEC (Hb) >500 mg/kg/day NOEC (%PCV) >500 mg/kg/day NOEC (RBC) >500 mg/kg/day NOEC (reticulocyte count) not estimated NOEC (MetHb) = 25 mg/kg/day NOEC (SulpHb) = 3 mg/kg/day (calculated with regression analysis) NOEC (spleen weight) >500 mg/kg/day	Keet et al. 1982
Sheep	0, 500, 2500 and 10,000 mg/kg in feed for 13 weeks.	No treatment-related effects were observed on food consumption, body weight gain, hematological parameters or urinalysis. Increase in MetHb and SulfHb and a reduction in the weight of the thyroid.	Ross et al. 1977

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Chronic Oral			
Dogs, beagle, 5/sex/dose group	daily oral administration of 0, 2, 10, 50, or 250 mg/kg/bw technical grade, air-milled diflubenzuron via gelatin capsules, 7 days/week for 52 consecutive weeks.	There were no clinical signs of toxicity, no treatment-related effects on body weight, food consumption, or water consumption; no ocular effects; there were treatment-related *marginal but statistically significant* increases in met Hb% and sulph Hb% (at ≥10 mg/kg/day bw) and in Heinz body counts (at 50 and 250 mg/kg/day bw); there was a marginal but consistent compound-related decrease in MCHC (at ≥10 mg/kg/day bw); histopathological changes included increased spleen weight (statistically significant in males at ≥50 mg/kg/day bw), increased liver weight (significant at ≥50 mg/kg/day bw in males and females) and hemosiderin deposition in the liver. The investigators conclude: *the no effect level demonstrated...was 2 mg/kg/day. However, this level is based on minor hematological changes of no toxicological significance seen at 10 mg/kg/day. Hence it is more realistic to consider the no effect level based on organ weights and histopathology as being at least 10 mg/kg/day.* Mortality: 2 females dogs died during the study. One dog at 250 mg/kg/bw) was sacrifice *in extremis* at week 33 due to liver failure and the other dog (at 50 mg/kg/day bw) died during week 40 due to bronchopneumonia. These effects were not attributable to treatment.	Greenough et al. 1985 MRID 00146174 **[This study is the basis for the chronic RfD]**

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Mice, CFLP, approximately 8 weeks old, 36/sex/dose group	In diet concentrations of 16, 80, 400, 2000, or 10,000 ppm (**intake values = 1.24, 6.40, 32. 16, 163.29, or 835.55 mg/kg/day for males and 1.44, 7.26, 35.38, 186.59, or 958.51 mg/kg/day for females**) technical grade DFB (97.6% pure) for 91 weeks.	Treatment-related clinical sign of toxicity was a blue/gray discoloration of the extremities and dark eyes in all mice at 10,000 ppm, a majority of mice at 2000 or 400 ppm, and in a number of mice at 80 ppm. The NOEC for this effect =16 ppm. No obvious treatment-related effect on mortality was observe; no obvious treatment-related effect on food consumption, body weight, food efficiency, or water intake was observed; treatment-related changes were principally associated with oxidation of the haemoglobin or with hepatocyte changes. DFB is not carcinogenic to DFLP mice.	Keet et al. 1984b
Mice, 88/sex/dose group	in diet concentrations of 0, 16, 80, 400, 2000, or 10, 000 ppm 97.6% pure difllubenzuron for 91 weeks. The **calculated mean intake** of diflubenzuron was 1.24, 6.40, 32.16, 163.29, or 835.55 mg/kg/day (males) and 1.44, 7.26, 35.38, 186.59, or 958.51 mg/kg/day (females) [*cf* page 47]	No treatment-related mortality throughout the study, no evidence of tumorigenic effect; treatment-related effects were primarily associated with oxidation of haemoglobin (**treatment-related increases in Met Hb% were recorded from week 26 onwards and in Sulph Hb% from week 52 onwards; these changes principally affected mice at 80-10,000 ppm and were dose-related in degree**) or with hepatocyte changes (**an increased incidenc of hepatocyte enlargement was observed in males and females at 400-10,000 ppm**).	Colley et al. 1984 MRID 00142490
Rats, Sprague-Dawley, 50/sex/dose group	in diet concentrations of 0, 156, 625, 2500, or 10,000 ppm technical grade diflubenzuron (97.6% a.i.) for 104 weeks.	No treatment related effects with regard to mortality or clinical observations; no evidence of carcinogenicity after 2 years of dietary exposure to diflubenzuron; statistically significant dose-related increases in met Hb% and sulph Hb% in males and females; numerous hematological effects; histomorphological changes observed in sections of the spleen, liver, and bone marrow; in general adverse effects were most pronounced at the 2500 and 10,000 dose levels.	Burdock 1984 MRID 00145467

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Rats, Sprague-Dawley, approximately 7 weeks old, 50/sex/dose group	In diet concentrations of 156, 625, 2500, or 10,000 ppm **(intake values =6.99, 28, 36, 114.35 or 463.80 mg/kg/day for males and 9.23, 37.98, 153.96, or 633.41 mg/kg/day for females**) technical grade DFB (97.6% pure) for 104 weeks.	No treatment related clinical signs observed; no obvious treatment-related effect on mortality; no obvious treatment-related effect on food consumption or body weight, except in high dose females where terminal body weight was significantly less than controls; no evidence of tumorigenic effects, treatment-related changes were principally associated with oxidation of haemoglibin or with hepatocyte changes. DFB is not carcinogenic to Sprague-Dawley CR-CD rats.	Keet et al. 1984a

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Reproduction Studies			
Rats, Crl:CD(SD)BR	0 or 1000 mg/kg bw per day on days 6–15 of gestation	Screening assay for teratogenicity. No signs of developmental toxicity, birth defects or maternal toxicity.	Kavanagh 1988a
Rabbits, New Zealand White	0 or 1000 mg/kg bw per day on days 7–19 of gestation	Screening assay for teratogenicity. No signs of developmental toxicity, birth defects or maternal toxicity.	Kavanagh 1988b
Rats, Charles River 32/sex/dose group	in diet nominal concentrations of 0, 500, 5000, or 50,000 ppm technical diflubenzuron through two consecutive generations. F_0 generation mean intake values (weeks 1-10 *pre-mate*) were 36.2, 360, or 3755 mg/kg/day for males and 42.0, 427, or 4254 mg/kg/day for females. F_1 generation mean intake values (weeks 5-16 *pre-mate*) were 39.2, 394, or 4089 mg/kg/day for males and 44.9, 473, or 4611 mg/kg/day for females	No treatment-related morality; toxicity manifested as hematological effects characterized primarily by anemia and increases in MetHb% associated with increased spleen weight and pathological lesions of hemosiderosis of the spleen and brown pigmented Kupffer cells in the liver were observed all dose levels. Increases in MetHb ranged from about 115% in the low dose group to over 300% in the high dose group (see Section 3.3 for more complete discussion and details). Other treatment related effects on the parental rats included lower body weight gains of the F_0 generation at 50,000 ppm, with higher food intake values in males; increased water consumption among males and females at 5000 or 50,000 ppm and among males at 500 ppm. No treatment-related effects on reproductive performance at any dose level. In the F_1 generation, liter and mean pup weights of the offspring from parents in the 50,000 dose group were lower than controls. The effect was not observed in the F_2 offspring. NOEL = 50,000 ppm for reproductive function NOEL = 5000 ppm for pre-weaning development of the offspring. NOEL = >500 ppm for MetHb	Brooker 1995 MRID 43578301 NOTE: U.S. EPA (1996) appears to classify the low dose group as the LEL for MetHb but specifies the dose as 25 mg/kg/day. This error appears to be based on the use of default values for converting food concentrations to mg/kg/day doses.

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
DERMAL			
Rabbits, New Zealand white, 5 males and 5 females	Dermal application of 5000 mg/kg Dimilin 2L (22.36% pure) to closely clipped intact trunks (approximately 10% of the body surface area). Treated area covered with gauze and occlusive wrap for 24 hours.	No mortality; no pharmacological or toxicological signs of toxicity; no severe dermal effects; no abnormalities observed during postmortem macroscopic evaluation. NOEC = 5000 mg/kg	Blaszcak 1997b MRID 44574505
Rabbits, New Zealand white, 4 males and 2 females, young adults, 2.2-2.6 g	Dermal application of 0.5 mL Dimilin 2L (22.36% pure) to intact skin of backs (hair closely clipped). Test site was semi-occluded with gauze for 4 hours	4/6 rabbits had slight, barely perceptible, erythema; 1/6 had slight erythema; 1/6 had no signs of dermal irritation. Dimilin 2L considered *slightly irritating* (FIFRA Primary Irritation Index = 0.5)	Blaszcak 1997d MRID 44574508
Guinea pigs, Dunken Hartley, 10/sex	Induction dose of approximately 0.3 mL Dimilin 2L (22.36% pure) for 6 hours; challenge dose after 14 days with 100% test material	No dermal sensitization responses during induction or challenge phase.	Blaszcak 1997e MRID 44574509
Rats. Charles River, 10/sex/dose group, weight = 284-314 g (males) and 201-233 g (females)	Dermal application of 20, 500, or 1000 mg/kg/day Dimilin (technical diflubenzuron) to shaved intact skin for 21 days.	No treatment-related effects on survival, clinical signs of toxicity, dermal observations, body weights, food consumption or macroscopic and microscopic pathology. Females in the 500 and 1000 mg/kg/day group had mild but statistically significant decreases in mean erythrocyte counts, hemoglobin, and hematocrit values; males in the 1000 mg/kg/day group had mild but statistically significant decreases in mean hemoglboin and hematocrit values. At 500 and 1000 mg/kg/day, males and females had an increased incidence of polychromasia, hypochromasia, and anisocytosis. At 1000 mg/kg/day, males and females had mild but statistically significant increases in Met Hb values and males also had mildly increased Sulph Hb values. NOEL = 20 mg/kg/day.	Goldenthal 1996 MRID 43954100-01

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Rats, Sprague-Dawley, males, 12/dose group	single dermal applications of ^{14}C-diflubenzuron suspended in 0.25% (w/v) gum tragacan WLC-grade water at 0.005 or 0.05 mg/cm^2 to shaved skin for periods of 1, 4, and 10 hours.	> 89% of the applied dose was removed by washing; 6% of the applied dose was found in the skin and increased exposure time did not increase the percent of dose found in the skin, although the amount of test material found in the skin was nearly proportional to dose; blood, carcass, and excreta accounted for negligible amounts of the applied dose; systemic absorption, excluding the skin was <1% of the total applied dose. These data indicate that the material that was absorbed was absorbed quickly. and the percent of applied dose that was absorbed appeared to be constant regardless of dose.	Andre 1996 MRID 44053101
EYES			
Rabbits, New Zealand white, 6	0.1 mL Dimilin 2L instilled in lower conjunctival sac of the right eye of each rabbit. Observations for ocular irritation made at 1, 24, 48, and 72 hours.	Positive scores (slight to moderate conjunctival irritation) in 3/6 rabbits within 24 hours of exposure with full recovery within 48 hours. No signs of iridial or corneal changes. The remaining 3 rabbits did not have positive scores for ocular irritation at any time during the study. Study demonstrates that Dimilin 2L is an "eye irritant" based on the results of positive scores in 3/6 animals with all changes being reversible.	Blaszcak 1997c MRID 44574507
INHALATION			
Rats, Sprague-Dawley, approximately 6-weeks old, 10/sex/dose group	Nose-only exposure to 0, 10, 30, or 100 mg/m^3 Dimilin technical 6 hours/day, 5 days/week for 4 consecutive weeks.	Dimilin technical produced minimal toxicity, including a slight (5-7%) decrease in erythrocytes, slight statistically significant decreases in hemoglobin and hematocrit in males and females at 100 mg/m^3 and an increase in bilirubin in males at 100 mg/m^3. No treatment-related effect observed on methemoglobin. NOEC = 30 mg/m^3	Eyal 1999 MRID 44950601

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Rats, Sprague-Dawley, 9 weeks old, 5 males (323-335 g) and 5 females (234-249 g)	4-hour nose only exposure to 2.0 mg/L Dimilin 2 L (22.36% pure) with 14-day post exposure observation period	No mortality; signs of toxicity during exposure included red nasal discharge and labored breathing; chromodacryorrhea, red nasal discharge, and excessive salivation, labored breathing, and moist rales were observed in some rats up to 1 day after exposure with complete recovery thereafter; slight weight loss was observed in some females during the first week after exposure followed by complete recovery during the second week; no abnormal macroscopic effects were observed during postmortem evaluation. LC_{50} >2.0 mg/L	Hoffman 1997 MRID 44574506
Rats, Sprague-Dawley, 20 males and 20 females, 5/sex/dose group	Whole body exposure to nominal concentrations of 0.5, 5.0, or 50 mg/L air 5 days/week for 3 weeks. Corresponds to 500, 5000, and 50,000 mg/m^3 – i.e., 1000 L = 1 m^3.	No signs of irritation at 0.5 mg/L; frequent blinking and occasional bouts of persistent sneezing and slightly labored breathing during exposures to 5.0 mg/L, followed by rapid recovery between exposures; at 50 mg/L, the signs observed in the mid-dose group were more pronounced and more persistent but repeated exposure did not result in cumulative adverse effects and recovery was rapid after each exposure period. No changes in body weight, compared with controls and no effects on water or food consumption were observed. Post-exposure **methaemoglobin** levels were increased 0.2-0.5 g% over controls (0.1 g%). The increase was statistically significant in the mid and high-dose males and in all treated females.	Berczy et al. 1975 MRID 00044325

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Rats, Sprague-Dawley, males and females, 6-weeks-old, 10/sex/dose group	Nose-only exposure to 0, 10, 30, or 100 mg/m^3 (measured as 12, 34, or 109 mg/m^3 diflubenzuron technical (95.6% purity) 6 hours/day, 5 days/week for 4 weeks.	Minimal toxicity: slight decrease (5-7%) in erythrocytes, slight statistically significant decreases in hemoglobin and hematocrit in males and females at 100 mg/m^3; increase in bilirubin males at 100 mg/m^3. A reduction in 'grid count' was evident in a neuro-functional assessment at the highest concentration. No effect observed on methemoglobin. NOEC = 30 mg/m^3	Newton 1999 MRID 44950601
		4-chloroaniline	
Rats, Wistar, SPF albino, males and females, 10/sex/dose group	daily oral doses of 0, 8.0, 20.0, or 50.0 mg/kg **4-chloroaniline (4-CA)** for 3 months	All rats at 50 mg/kg had increased numbers of Heinz bodies (>20/100 RBC) and a reticulocyte response (>2%); however there was no evidence of a decrease in hemoglobin, packed cell volume, or RBC count. Histological changes were observed only in the high dose group and included increased extramedullary haematopoiesis in spleen and liver and occasionally in the lung; increased hemosiderin (from hemoglobin breakdown) in the liver and spleen and occasionally in the kidneys (epithelium of proximal convoluted tubules). NOEC = 8.0 mg/kg	Scott and Eccleston 1967

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Dog, Beagle, males and females, 4/sex/dose group	daily oral doses of 0, 5, 10, or 15 mg/kg **4-chloroaniline (4-CA)** for 3 months	One dog in the 15 mg/kg dose group died as a result of excessive haemolysis (after receiving 25 mg/kg 4-CA). 5/7 remaining dogs receiving 15 mg/kg 4-CA showed an early and marked decrease in RBC count (>1.5 M) and packed cell volume (>15%) with a concomitant decrease in hemoglobin levels. The same trend was observed in half the dogs at 10 mg/kg and one of the dogs at 5 mg/kg. Lowest levels of RBC and hemoglobin were reached at approx 3-4 weeks, after which time, there was a slow but steady improvement in all values, despite the persistence of increased numbers of Heinz bodies. A reticulocyte response and an in crease in Heinz bodies were observed in all dogs at 15 mg/kg, most dogs at 10 mg/kg, and three dogs at 5 mg/kg, while the control group remained normal. All treated dogs showed histological changes, including evidence of hematopoietic response in extramedullary activity in spleen and liver at all doses (The marrows showed hyperplasia of the erythroid phase) and marked evidence of RBC destruction in the spleen, and liver.	Scott and Eccleston 1967
Rats, Fischer 344, males, 10/dose group	In diet concentrations of 1240 ppm **4-chloroaniline** or 1240 or 4320 ppm **p-chlorophenylurea** for 7 days	1240 ppm **4-chloroaniline** caused statistically significant increases in methemoglobin values at all intervals of analysis No treatment related effects on methmoglobin values in rats treated with 1240 or 4320 ppm **p-chlorophenylurea**. The only macroscopic change observed was enlargement of the spleen in rats from the 1240 ppm **4-chloroaniline** group. No mortality.	Goldenthal 1999b MRID 44871303

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Rats, Fischer 344, approx 6-weeks old, males and females, 25/sex/dose group	In diet concentrations of 250 or 500 ppm **4-chloroaniline** for 78 weeks with a 24-week observation period.	Mean body weight depression associated with treatment was observed in high dose females, compared with controls. No significant treatment-related mortality among females; however, there was a significant (p=0.0294) correlation between dose and mortality in males rats. In the high dose male rates, the incidence of unusual splenic neoplasms (i.e., fibroma, fibrosarcoma, sarcoma, hemangiosarcoma, and osteosarcoma) was increased (0/20 controls; 0/49 low dose, 10/49 high dose). This finding was considered strongly suggestive of carcinogenicity because of the rarity of the rumors in the spleens of controls rats. Formation of non-neoplastic lesions of the splenic capsule in rats in all dose groups.	NCI 1979
Mice B63CF1, approx 6-weeks-old, males and females, 25/sex/dose group	In diet concentrations of 2500 or 5000 ppm **4-chloroaniline** for 78 weeks with a 24-week observation period	Mean body weight depression associated with treatment was observed in all mice, compared with controls. No significant treatment-related mortality in mice of either sex.	NCI 1979

Appendix 2: Laboratory and simulation studies on environmental fate of diflubenzuron and its metabolites.

Data Summary	Reference
Aquatic Sediments	
anaerobic aquatic metabolism of 1.3 mg/kg ^{14}-C diflubenzuron in silt loam/water system. DT_{50} = 34 days for total hydrosoil/water system and 18 days for water-phase only. 2,6-difluorobenzoic acid, and 4-chlorophenylurea were main metabolites that accumulated in the anaerobic water phase; hardly any bound residue detected.	Thus et al. 1991 MRID 41837601
two model ditch (water/sediment) systems (sandy loam or silt loam covered with surface water) with addition of 0.94 ppm. diflubenzuron with continuous flow through upper layer of surface water. Incubation at 20±1° w/12 hour photo period. Results indicate rapid disappearance of compound from model ditch systems due to rapid metabolism and adsorption to sediment. Water phase DT_{50} = 1.1 day (silt loam) and 1.9 days (sandy loam). Complete sediment/water systems DT_{50} = 10 days (silt loam/surface) and 25 days (sandy loam/surface). Only metabolites were DFBA and CPU	Thus and van der Laan-Straathof 1994 MRID 44399307
0.013 ppm DFB in a microbially viable soil/water test system DFB was readily degradable under aerobic aquatic conditions half-life (first-order kinetics) = 25.7 days (r^2=0.709) DT_{50} = 5.3 days major metabolite formed, 4-chlorophenylurea half-life (first-order kinetic) = 39.7 days (r^2=0.671)	Dzialo and Maynard 1999 MRID 44895001
degradation rate of 50 µg/L diflubenzuron in seawater in the presence of salmon feces and sediment is temperature dependent: at 15°, DT_{50} = 3 ½ weeks (anaerobic) or 4 ½ weeks (aerobic); however at 5°C, there was no significant difference between the anaerobic (DT_{50} = 99 days) or the aerobic (DT_{50} = 100 days) test conditions. The metabolites included 4-chlorophenylurea, 2,6-difluorobenzoic acid, 2,6-difluorobenzamide, and CO_2	van der Laan 1995 *In*: Technology Sciences Group 1998 MRID 44399307

Appendix 2: Laboratory and simulation studies on environmental fate of diflubenzuron and its metabolites.

Data Summary	Reference
laboratory microcosm study using 10 µg/L DFB in seawater with or without sediment. half-life of DFB in seawater **without** sediment = 18.7 days half-life of DFB in seawater **with** sediment = 5.2 days presence of organic sediment in DFB-treated microcosm significantly reduced the efficacy of DFB in seawater as measured by toxicity of aged DFB (initial nominal concentration of 10 µg/L) to 5-day old grass shrimp embryos. By day 30, embryos reared in seawater from DFB-sediment microcosm produced larvae with no signficant morphological abnormality and larval viability was comparable to controls; embryos reared in DFB-treated seawater **without sediment** produced larvae with severe abnormalities and very low viability even after the seawater aged for 65 days.	Wilson et al. 1995
persistence of diflubenzuron (Dimilin) in sod-lined water pools after repeated applications: Bioassay data indicate toxicity greatest during the first 24 hours; DFB fell below detection limits (1µg/L) within 24 hours, whereas chlorophenylurea concentration increased for several days after treatment.	Madder and Lockhart 1980
Bioconcentration	
Channel catfish, *Ictolurus punctatus*: No bioconcentration. In 0.01 ppm tanks, concentration in muscle was below 0.002 ppm and concentration in viscera peaked at about 0.003 ppm (Figure 2). Similar pattern in 0.5 ppm tanks (Figure 3). **Algae:** BCFs of 2412 at hour 1 to 109 at day 4. Probably reflects degradation – i.e., algae degraded 80% of the DFB in a 1-hour incubation period.	Booth and Ferrell 1977
Laboratory algae culture system of *Scenedesmus subspicatus* exposed at an initial concentration of 200 µg/L DFB for 7 days no growth inhibition; half-life = 3 days DFB radioactivity in algae increased steadily and leveled off at approx. 60% after 5 days BCF values decreased from 4310 to 889 during the exposure period elimination was rapid during the first hours.	Yu-Yn et al. 1993
Hydrolysis	
rapid decrease in of residue levels. Half-life w/aeration = 0.41899 days (tap water and natural sunlight) Half-life wo/aeration = 0.96685 days (tap water and natural sunlight)	Anton et al. 1993

Appendix 2: Laboratory and simulation studies on environmental fate of diflubenzuron and its metabolites.

Data Summary	Reference
Two applications of Dimilin 25W (25% a.i. by weight) to surface of littoral enclosures using portable hand sprayer at rates of 4-210 g/ha. Maximum residues in water column measured withing first 24 hours after application, Half-lives ranged from 3.28 to 8.23 days with a mean of 4.28 days. By 14-35 days (or a mean of 18.5 days), 95% of the diflubenzuron dissipated. Principal loss from water column early in the study probably due to adsorptive processes because temperature and pH were not favorable for rapid aqueous hydrolysis.	Knuth 1995 MRID 44386201 (This is chapter 2 of Moffet 1995)
11 µg/L ^{14}C-diflubenzuron in a CO_2-evolution test (concentration below aqueous solubility). DT_{50} = approximately 2.5 days; hydrolysis products are DFBA, CPU, and CO_2	van der Laan and Thus 1993 *In*: Technology Sciences Group 1998 MRID 44399307
High temperature (121˚C) increased the degradation of diflubenzuron in aqueous media at levels greatly above its solubility in water and resulted in its rapid degradation to as many as seven identified products: 4-CPU, 2,6-DFBA, 2,6-difluorobenzamide, 4-chloroaniline, *N,N'*-bis (4-chlorophenyl) urea, 1-(4-chlorophenyl)- 5-fluoro-2,4 (1H,3H)-quinazolinedione and 2-[(4-chlorophenyl) amino]- 6-fluorobenzoic acid. 4-Chloroaniline, *N,N'*-bis (4-chlorophenyl) urea and 2[(4-chlorophenyl) amino]-6-fluorobenzoic acid were not detected at lower temperatures (0.1 mg [^{14}C]-diflubenzuron/L water or buffer at 36˚C). 4-Chloroaniline was a major degradation product of diflubenzuron in heat-treated samples, but it was not seen at lower temperatures	Ivie et al. 1980

Photolysis

Photodegradation half-lives of diflubenzuron in deionized water (pH 7) = 17 hours; in deionized water (pH 9) = 8 hours; and in river water (pH 9) = 12.3 hours. In a solar simulator using river water buffered to pH 9.0, the half-life for diflubenzuron =12 hours; dark controls showed no loss of parent compound over similar time periods. Log Kow = 3.8 (determined using reverse phase HPLC)	Marsella et al. 2000

Appendix 2: Laboratory and simulation studies on environmental fate of diflubenzuron and its metabolites.

Data Summary	Reference

Residues on Plants

persistence of diflubenzuron (commercial grade 25% WP) on Appalachian forest leaves. Leaves sprayed in spring and left to weather during growing season. white oak leaves collected in July and August and placed in headwater stream to monitor residual diflubenzuron showed rapid decrease in residue (36% in July and 23% in August) within the first 48 hours of stream incubation, reaching less than 10% of the original concentration within 3 weeks. Yellow poplar, read maple, and white oak leaves collected in December and place in headwater stream showed a much slower rate of loss. After 54 days in the stream, yellow poplar and red maple leaves retained 45 and 40%, respectively of the original diflubenzuron concentration and white oak showed no significant loss. Stream water temperatures averaged 17°C lower in December than in August (temperature readings were not made in July).	Harrahy et al. 1993

Soil Degradation/Transport

fate of 4-chloroaniline in nonautoclaved and autoclaved soil. in soil treated with 4-chloroanline and incubated for 6 weeks, no CO_2 evolution in occurred in autoclaved soil; in nonautoclaved samples, CO_2 was determined as 7.5% of the originally applied radioactivity.	Bollag et al. 1978
Cell suspension of 0.04 g *Pseudomonas putida* in 2 mL of 0.05 *M* phosphate buffer (pH 7.0) incubated with 10 µg ^{14}C-PH-6040 (DFB) (both A and B labels) for 6 hours produced no evidence of degradation upon extraction. Both labeled preparation were recovered intact as 99.9+% of total ^{14}C	Metcalf et al. 1975
10 ppm ^{14}C-PH-6040 (DFB) added to fresh, air-dried Drummer soil (17.4% moisture) and incubated at 80°F for 1, 2, or 4 weeks. Compound appeared to be very stable, with degradation products comprising only 0.7% of total extracted radioactivity after 4 weeks.	Metcalf et al. 1975
aerobic soil metabolism of 0.69 mg/kg ^{14}-C diflubenzuron in sandy loam DT_{50} = 50 hours; DT_{90} = 181 hours CO_2, 2,6-difluorobenzoic acid, and 4-chlorophenylurea were main metabolites; 2,6-difluorobenzamide and 4-chloroaniline were minor metabolites.	Walstra and Joustra 1990 MRID 41722801

Appendix 2: Laboratory and simulation studies on environmental fate of diflubenzuron and its metabolites.

Data Summary	Reference
10 ppm technical DFB applied on quartz sand to natural sandy loam and much soils at 12 weeks: 2-12% remaining (compared with 80-87% remaining in sterilized soil), indicating that soil microorganisms play a major role in the degradation of DFB. Kinetic analysis based on 1st order dependence indicates that the rate constants for disappearance reactions decreased with time.	Chapman et al. 1985
breakdown of DFB by soil isolates: *Rhodotorula* sp. half-life of detectable DFB = 18 days (carbon source: acetone) *Fusarium* sp. half-life of detectable DFB = 7 days (carbon source: DFB/acetone) *Penicillium* sp.half-life of detectable DFB = 14 days (carbon source: acetone) *Cephalosporium* sp.half-life of detectable DFB = 13 days (carbon source: acetone) Control half-life of detectable DFB = 27 days.	Seuffer et al. 1979
^{14}C-DFB readily degraded in various agricultural soils and in hydrosoil: 50% of applied dose (1 mg/kg) metabolized in ≤2 days. Chief products of hydrolysis were 4-chlorophenylurea and 2,6-difluorobenzoic acid.	Nimmo et al. 1984
initial dose of 1 mg/kg **4-chlorophenylurea** in decreased to 50% in about 5 weeks in aerobic sandy clay and in about 16 weeks in anaerobic hydrosoil Investigators assume that two sorts of bound residues are formed from **4-chloro-phenylurea**: one is fairly stable and might consist of bound 4-chloroaniline or its transformation products and the other is presumed to be a degradable derivatie of **4-chlorophenylurea**.	Nimmo et al. 1986
2-6-difluorobenzoic acid is rapidly and completely degraded in soil: time to 50% disappearance in 9 days in humus sand and after 12 days in sandy clay. DFBA completely disappeared in the humus sand after 32 days.	Nimmo et al. 1990
DFB (technical), Dimilin WP-25, and Dimilin SC-48 were applied separately at 70, 210, or 630 g ai./ha (corresponding to 17.23, 51.69, or 155.07 µg a.i.) To top layer of columns (30x5.6 cm id) packed either with sandy or clay loam forest soils. Mobility of DFB was low and did not increase with dosage. At deposit rate equivalent to 70 g a.i./ha, nearly all the residues were found with 2.5 cm of the top of the column. At 630 g a.i./ha, only about 9% of the technical DFB, 7% of Dimilin SC-48, and 4% of Dimilin WP-25 moved below the 2.5 cm level in sandy loam. No residues were found below the 10 cm level or in the leachates in either soil type at all dosage levels. In addition to soil type, mobility of DFB was also influenced by the additives present in the formulations with technical DFB > Dimilin SC-48 > Dimilin WP-25.	Sundaram and Nott 1989

Appendix 2: Laboratory and simulation studies on environmental fate of diflubenzuron and its metabolites.

Data Summary	Reference
Organic soil and silty clay loam soil collected from a boreal forest in northern Ontario, Canada maximum amount adsorbed: 88 µg/g (organic soil); 73 µg/g (silty clay loam) time required for maximum adsorption: 18 h (organic soil); 24 h (silty clay loam) Organic soil characterized as about equal parts sand, silt, and clay and 21% OM and 13% OC. Silty clay loam characterized as 22% sand, 49% silt, and 29% clay, and 8.2% OM and 5.1% OC. K_D = 17.59 (organic soil) K_D = 16.42 (silty clay loam) K_{oc} = 135.3 (organic soil) K_{oc} = 332.0 (silty clay loam) calculated K_{oc} = 144.4 (organic soil) calculated K_{oc} = 345.3 (silty clay loam)	Sundaram et al. 1997

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
33, 66, and 140 g a.i./ha (0.5, 1, and 2 oz a.i./ac)	No evidence of negative effects on predators/parasites – lacewing (*Chrysopa carnea*), ladybird beetle (*Hippodamia convergens*), Wasp parasite *Trichograma pretiosum* of bollworm (*Heliothis*). Immigration from untreated fields could mask negative effects on beetles seen in lab (see Appendix 5).	Ables et al. 1977
280 g a.i./ha (4 oz a.i./ac)	Caged lacewing suffered increased mortal. eating treated eggs. No effect on parasitic wasp through 2-3 generations; wasp developed in treated eggs and in eggs produced by treated adults, and direct exposure to adults was not toxic.	Ables et al. 1977
187 ppm spray to apple orchards (NOS)	No adverse effects in Phytoseiid and stigmaeid mites No population increases following treatment in European red and rust mites	Anderson and Elliott1982
Application (spray) of Dimilin WP, 0.6 kg in 600 L/ha to a 2.4 ha apple orchard (integrated pest management program). 250 g Dimilin/ha, 62.5 g a.i./he	DFB persisted on foliage until leaf-fall and was detected on the peel of harvested fruit. Mean residue on harvested Worcester fruit = 0.05 mg/kg fresh weight and on harvested Cox fruit, mean residue = 0.02 mg/kg fresh weight.	Austin and Carter 1986
4-year field study (1992-1995) in apple orchards in a codling moth control program based on 4 seasonal sprays/year. Diflubenzuron at 3-12 g/100 L. Application rate in g/ha not specified.	Spider fauna (26 genera and 30 identifiable spider species) in apple orchards of Western Oregon. DFB was harmless to spider species tested ($p>0.05$)	Bajwa and AliNiazee 2001
Dimilin 4 liquid applied at 70 g a.i./ha to watersheds in a central Appalachian broadleaf forest	Yellowjackets, (10 species of wasps, Family Vespidae): Diflubenzuron decreased worker number during application year but not in post application year. There was no effect of trap site on worker sample size.	Barrows et al. 1994
140 g a.i./ha (2 oz a.i./ac). 4.05 ha in 41 ha woods.	Some species of soil mites were adversely affected. Half the number in treated v. untreated samples.	Blumberg 1986
67 g a.i./ha (0.96 oz a.i./ac)	Wasp parasite on Gypsy moth eggs (*Ooencyrtus kuvanae*) on gypsy moth. Egg masses in treated plots were parasitized as heavily as egg masses in control plots. Lab data showed no effect on development and emergence from treated eggs or from eggs laid by treated adults.	Brown and Respicio 1981

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
350 g a.i./ha (5 oz a.i./ac)	5 hives (Honey bee, *Apis mellifera*.) in treated and untreated sites. No effects on egg hatch, brood production, numbers of adults, and honey production.	Buckner et al. 1975
70 g a.i./ha (1 oz a.i./ac)	Under tree bands, Carabidae (beetles), Gryllacrididae (grasshoppers), and two families of moths were significantly reduced in total taxa richness and abundance on treated sites.	Butler 1993

Additional Notes on Butler 1993: Foliage sampling found reduced abundance and richness in the following groups: Lepidoptera, Symphyta (sawflies, horntails), some herbivorous Coleoptera (beetles), Psocoptera (book lice, wood lice), predatory Thysanoptera (thrips), some Homoptera (leaf hoppers, aphids, cicadas), Diptera (flies), Orthoptera (grasshoppers), and Arachnida (spiders). Some affected by direct toxicity and others (predators) indirectly through prey reduction.

Aerial application of Dimilin 4L (35.1 g a.i./ha) to two watersheds in a Central Appalachian forest; two untreated watersheds served as controls.	Gypsy moth larvae decreased in number on the treated watersheds, especially during the treatment and post-treatment year. Macro lepidoptera larvae also decreased in number during the treatment year.	Butler et al. 1997 Butler 1995

Additional Notes on Butler et al. 1997: In treated watersheds, there was an overall reduction in arthropod family diversity and abundance on foliage and a significant reduction in the number of macro Lepidoptera and beetles. 27 months after treatment, total arthropod abundance and macro lepidoptera abundance on foliage remained significantly reduced. Decreases in the numbers of Carabidae (ground beetles), Gryllacrididae (crickets), Psocoptera (booklice/barklice), Phlaeothripidae (alligatorweed thrips), and some sapfeeders were observed but reductions were not significant.

Aerial application of 0.0084, 0.0168, or 0.0336 kg a.i./ha Dimlin 2F [8.4, 16.8, 33.6 g/ha] or 0.0168 kg a.i./ha Dimilin 25W [16.8 g/ha] to mixed-grass rangelands near Amidon, ND (experimental plots were 0.4x0.4 km).	Abundance of ants was not significantly reduced by treatment at any levels. Ant diversity declined temporarily (13-19 days) after treatment with Dimilin 25W, but recovered immediately the following week and no further declines were observed. Twenty species of ants were encountered in the experimental site.	Catangui et al. 1996
Aerial application of diflubenzuron (25% WP) to treatment plots at a rate of 70.75 g/ha on May 8, 1985 and May 9, 1986 as part of Gypsy moth suppression program in WV. Plots were located in an 8000 ha oak-hickory forest. Untreated plots served as controls.	**Abundance**: No significant differences were observed ($p<0.10$) in abundance of 21 species of birds examined between treated and control plots. **Diets**: All species in untreated plots ate more Lepidoptera larvae than species on treated plot; difference was significant ($p<0.10$) in 5 of 7 species. **Foraging**: Vireo foraging areas were 3.1 and 2 times larger on treated areas, compared with untreated areas.	Cooper et al. 1990

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
70 g a.i./ha (1 oz a.i./ac) to cotton, applied in paraffinic crop oil (Dimoil) and water. Sampling took place 1 week after each treatment. Fields 15 ha each.	Assay of populations of predators of bollworms (*Heliothis*): lacewings (*Chrysopa* spp.), ladybird beetle (*Hippodamia convergens*), *Coleomegilla maculata* big-eyed bug (*Geocoris punctipes*), *Nabis* spp., *Orius insidiosus*. Numbers of predators unaffected by 4 treatments 1 week apart. The study did not look at parasite numbers. The authors note that crop oil could have affected some species.	Deakle and Bradley1982
0.3 to 3.3 kg Dimilin 25W/ha [75 g a.i. to 825 g a.i./ha]	No effect on breeding success or growth of nestlings for tree sparrows (*Passer montanus*) or two species of tits (*Parus major* and *Parus caeruleus*). Endpoints examined included number of occupied nest boxes, mean number of offspring, nesting period, mortality of nestlings, and breeding success.	De Reed 1982
110 to 400 g a.i./ha	Honey bee, *Apis mellifera*. No effect from spray on trees on adults or larvae.	Emmett and Archer 1980
38 and 83 g a.i./ha applied in diesel oil (0.54 and 1.19 oz a.i./ac)	Nearly 90% reduction in grasshoppers (nymphs and adults) 7 d. after treatment at higher rate. Low rate had minimal effects on larval grasshoppers. At least one taxon of beetle showed reductions of 50% at highest dose. Possible reduction in trap catches of members of 1 of 3 families (the Gnaphosidae) of ground spiders, at highest dose, 4 weeks after treatment. Reduced populations of Ichneumonids and Braconids in sprayed plots for at least 3 weeks. Possibly due to effects on host species rather than direct toxicity. Tiphiids unaffected by treatments. Predatory wasp reduced in treated plot, possibly a response to prey reduction (grasshoppers).	Everts 1990
Brazil: 250 g a.i./ha (3.6 oz a.i./ac). Applied 3x by mistblower.	No effect on adult levels of predator *Calosoma*, nor on nabids or geocorids.	Heinrichs et al. 1979
70 g a.i./ha (1 oz a.i./ac) in 4.7 l/ha crop oil (Savol) + H2O, applied 6x at 5 d. intervals	Treatments reduced parasitism by *Trichogramma pretiosum* to *Heliothis* spp. by 44% after spray.	House et al. 1980
Apple orchard in Union, CT. 57 g a.i./10 gal water with spreader sticker. Applied with backpack sprayer.	Parasitic wasp *Apanteles melanoscelus* Parasitism rate on treated vs. control trees roughly equal before spray, but lower on treated trees 7 d. after spray (1.81% v. 0.67%). Some adult wasps developed successfully, perhaps those in later stages of development.	Granett and Dunbar 1975

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Apple orchard in Brooklyn, CT. 3.5 g a.i./10 gal. water with spreader sticker. Applied w/ backpack sprayer.	Parasitic wasp *Apanteles melanoscelus* 1st application of spray decreased parasitism rate. 2nd and 3rd applications did not.	Granett and others1976
About 11 and 22 g a.i./ha (0.75% and 1.0% a.i./kg. At 1.1 and 2.2 kg/ha.) Treated bran bait.	30+ spp. of grasshoppers, counted on treated and control fields. Total populations were reduced 28 days after treatment by 60 and 70% at highest rates of application (0.75 and 1.0% a.i./kg; 2.2 kg/ha). Populations reduced <20% at half that rate. Greater effects early instars.	Jech et al. 1993
Cotton fields treated with nine applications of 2 oz a.i./acre (140 g/ha) diflubenzuron (NOS) from June17-Aug12	Monitoring of arthropod predator populations: *Geocoris punctipes*, *Nabis* spp., *Hippodamia convergens*, *Coleomegilla maculata*, *Orius insidiosus*, *Chrysopa* spp. Diflubenzuron treatment did not skew the relative abundance of the predators sampled. For 6 days after collection, egg hatch in the laboratory held *H. convergens* was significantly lower in females collected from treated cotton fields, compared with those form untreated cotton fields.	Keever et al. 1977
Backpack application of 8 oz Dimilin 25W or 0.5 pints Dimilin 2L (0.125 lbs a.i./acre in each case) to maturing cotton foliage in Fresno, CA or East Bernard TX	Over 5 weeks, dislodgeable foliar residue ranged from 0.40 µg/cm^2 down to 0.01 µg/cm^2 (limit of quantitation). Regression analysis predicted mean dislodge able residues on cotton leaves of 0.189 µg/cm^2 at 4 hours and 0.180 µg/cm^2 at 24 hours at both locations.	Korpalski 1996a MRID 44081401
Three applications of Micromite 25W via calibrated airblast sprayer to orange trees at a rate of 1.25 lbs (0.3125 lbs a.i./acre) in LaBelle, FL. [0.35 g/ha × 3]	Over 5 weeks, dislodgeable foliar residue ranged from approximately 0.8 to 1.0 µg/cm^2 shortly after the last application and down to 0.22 to 0.48 µg/cm^2 at 35 days post application. Regression analysis predicted mean dislodgeable residues on orange tree leaves of approximately 0.59-0.82 µg/cm^2 at 4 hours and approximately 0l58-0.81 µg/cm^2 at 24 hours at both locations.	Korpalski 1996b MRID 440814012
Diflubenzuron at 150, 450, or 750 g a.i/ha.	Gram pod borer, *Helicoverpa armigera* (Lepidotera: Noctuidae) [crop pest] field collected eggs on gram plants in sprayed and unsprayed plots. % egg mortality: Controls = 13.0% ; 150 g/ha. =39.0%; 450 g/ha. = 61.0%; 750 g/ha. = 100.0 %	Kumar et al. 1994

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Site 1: 140 g a.i./ha (2 oz a.i./ac) Site 2: 280 g a.i./ha (4 oz a.i./ac) both in Kamloops, British Columbia	Mites counted in the top 6 cm of soil. About half of the taxa showed significant decreases in abundance from diflubenzuron applications. Overall population unaffected by spraying; increases in some species compensated for decreases in others. Mites in upper 3 cm of soil more severely affected than mites below. Some predators decreased and some increased (trophic level not predictive of susceptibility). 4 species apparently eliminated from site 2, after a year; other species persisted at low levels a year after spray.	Marshall1979
34 and 68 g a.i./ha (0.5 and 0.97 oz a.i./ac)	Honey bee, *Apis mellifera*. Hives placed in gypsy moth treatment blocks. No effects from applications on numbers of adults, larvae, or honey production.	Matthenius1975
Aerial application of Dimilin 25-W at a rate of 70.75 g/ha (2 oz/acre) to 770x770 m (60 ha) plots on May 8, 1985. Plots were separated by at least 150 m to minimize the effects of spray drift. The study area (Morgan Co, WV) was characterized by mature oak-pine and oak-hickory forests. Gypsy moths were mostly 1st and 2nd instars and foliage was not fully expanded at the time of treatment.	Foliage residues: 1 day after treatment = 0.45±0.25 ppm 3 days after treatment =0.31±0.16 ppm 10 days after treatment =0.10±0.06 ppm 21 days after treatment =0.18±0.16 ppm	Martinat et al. 1987
Aerial application of Dimilin 25-W at a rate of 70.75 g/ha (2 oz/acre) to 770x770 m (60 ha) plots on May 8, 1985 and May 9, 1986 Plots were separated by at least 150 m to minimize the effects of spray drift. The study area (Morgan Co, WV) was characterized by mature oak-pine and oak-hickory forests.	Significant, treatment-related reductions were observed primarily in canopy macrolepidoptera and non-lepidopteran mandibulate herbivores. Sucking herbivorous insects, microlepidoptera, and predaceous arthropods were not affected.	Martinat et al. 1988
70 g a.i./ha (1 oz a.i./ac)applied to oak-pine and oak-hickory hardwood.	120 species of spiders (Araneae) and orthopteroid (Orthoptera and Dictyoptera). Significant effects from treatments noted on spider on 1 of 10 sampling dates, and on orthopteroid abundance on 2 of 10 sampling dates. Trend in expected direction on other dates. No change in diversity of these groups. Effect on spiders could be from loss of prey or direct toxicity. Orthropoids picking up from litter that they ingest.	Martinat et al. 1993

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Application of 280 g/ha a.i. Dimilin WP-25 via backpack sprayer to rice field 5 days after emergence of rice leaves out of the water.	Half-life (calculated from first order kinetics) = 27 hours; residues were below detection limit after 96 hours.	Mabury and Crosby 1996
Additional notes on Maybury and Crosby 1996: Sensitized photolysis was the primary route of degradation, although partitioning to sediment and volatilization may have played minor roles in the fate of the compound. Rapid photolysis of DFB to CPU and DFBA. This mixture was as toxic to daphnids as DFB. Photolysis in distilled water is slow. Halftime of alkaline (pH 8.8) photodegradation was 157 hours (2.4 days) and filtered field water (pH 7.4) was 32 hours (1.3 days). Slower rates of photodegradation for CPU and DFBA. Field dissipation halftime for DBF of 27.3 hours (1.1 days) with typical initial increase in concentrations of CPU.		
30 g a.i./ha, in 4.78 l water (0.43 oz a.i./ac)	Wasp parasite on gypsy moth larvae (*Apanteles melanoscelus*)Parasitic fly in family Tachinidae. Wasp mortality 80% in 2 weeks from field spray. Development halted in most cases, failed to spin cocoons upon emergence, etc. 100% mortality in tachinid parasite. Gypsy moths in 2nd, 3rd, and 4th instar.	Madrid and Stewart1981
Aerial (fixed-wing aircraft) application of Dimilin WP-25 at a rate of 75 g a.i. in 50 L water/ha in A total of 1160 ha of insect-infested forest in Finland in August 1984 in an effort to control the pine looper, *Bupalus piniarius.*A solution of hydroxyethyl cellulose and 15 g sodium bicarbonate in 50 L water/ha was added to formulation to minimize drift, especially near the borders of the treated area.	Residues in run-off water decreased from 5 µg/L one day after spraying to 0.1 µg/L after 2 months. The concentration in water in open pits was 0.1 µg/L 1 and 7 days after application and 0.2 µg/L 1 month after application. After 2 months no residues were detected. All water samples taken from outside the treated area contained < 0.1 µg/L (the limit of sensitivity). No DFB was detected in the treated area the year following application or outside the treated area. Neither 4-chloroaniline nor 4-chlorophenylurea was detected in the water at any time. Residue data for the litter layer, humus layer, pine needles, wild mushrooms, boletus samples, and bilberries are provided.	Mutanen et al. 1988
DFB (25% WP) via handgun to four-tree Valencia orange blocks at a rate of 10 oz a.i./acre. Trees were sprayed to runoff to control citrus rust mite.	Half-lives of DFB surface residues (Exp 1 cool-dry period: March to April): leaves =*essentially none* fruit =118±100 days; soil (middle) = 19±11 days; soil (dripline) = 21±10 days; Half-lives of DFB surface residues (Exp 1 hot-wet period: March to April): leaves + 27±8 days; fruit 18±2days; soil (middle) = levels too low to be detected; soil (dripline) = levels too low to be detected	Nigg et al. 1986

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Aerial application of 70 g a.i./ha Dimilin to experimental watershed (two treated; two controls) in the Fernow Experimental Forest, WV. Soil and leaf litter arthropods were monitored before and after application for a total of 36 months.	Throughout the study, mites (49%) and springrtails (28%) dominated the soil core sample. A total of 19 taxonomic groups were suitable for statistical analysis. No significant treatment effects were observed, based on total organism counts or counts by trophic categories ($p<0.05$).	Perry et al. 1997 Perry 1995b
Additional Notes on Perry et al. 1997: No significant treatment-related effects for populations of major taxonomic groups, except for Araneae (spiders) were observed. Analysis of leaf-litter bags also indicated no significantly differences in total numbers of invertebrates or in trophic categories between treated and untreated watersheds during the 12-month post treatment study. There appeared to be an indirect effect on spiders as a taxon, which may have resulted from changes in prey populations.		
Aerial application of Dimilin 25W at a rate of 33.23 g a.i./ha in 9.4 L/ha to a 20-ha forest block in central PA. Leaf samples were collected from the upper and lower canopies of 27 oaks and understory within the block on the day of application, May 29, 1991. Canopy leaves were also collected on May 31, June 10, July 29, and September 26, 1991.	On the day of application, DFB residues on the upper canopy, lower canopy, and understory averaged 81.18, 39.65, and 8.35 ng/cm^2. DFB residues on canopy leaf residues were: 14.83 ng/cm^2 (day 2 post spray) 16.75 ng/cm^2 (day12 post spray) 12.84 ng/cm^2 (day 61 post spray) 11.20 ng/cm^2 (day 120 post spray) DFB residues on litter-leaf sample collected after leaf senescence 169 and 323 days after treatment contained measurable amounts of DFB in 51 and 59% of the samples, respectively.	Prendergast et al. 1995
Three cover sprays of diflubenzuron (NOS) at 3.7 or 7.4 g a.i./100 L in a pear orchard in northern CA. [Data to calculate application rate in g/ha not given]	DFB treatment had no direct effect on pear psylla (pest species), did not induce phytophagous mites, and was weak, compared with the synthetic pyrethroid, fenvalerate against the codling moth.	Riedl and Hoying 1980
0.5 and 2 oz a.i./ac, w/ crop oil, sprayed 8 times on cotton. [35 to 140 g/ha × 8]	Direct spray of bee hives. No effects noted on adult mortality, rate of larval growth, brood production, or honey or wax production. No residues in wax or honey. Not caged study, so bees could have foraged outside of spray area.	Robinson 1978,1979
Aerial application of oil formulation of DFB (Dimilin 45 ODC) on August 31st in a conifer forest in the north of Spain at a dose of 56.3 g a.i./ha a(125 cm^3 Dimilin in 5 L diesel oil) (volume rate of application = 5 L/ha). The day of application was clear with no rainfall in the previous 48 hours.	DFB persisted for 10-12 weeks on the foliage of the conifer forest; 55-80% of the insecticide was removed from the foliage within 22-30 days after treatment; aerial application resulted in residue levels of 867.5-1824.4 ng/g, depending on the forest characteristics. 2,6-difluorobenzmide was the only metabolite detected and persisted only until the first rainfall.	Rodriguez et al. 2001

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Aerial application of Dimilin 25-W at a rate of 70.75 g/ha (2 oz/acre) to 770x770 m (59.2 ha) plots on May 9, 1986 Plots were separated by at least 150 m to minimize the effects of spray drift. The study area (Morgan Co, WV) was characterized by mature oak-pine and oak-hickory forests.	Diets of five species of forest birds were significantly different between treated and untreated plots. Treatment generally decreased the biomass of Lepidoptera larvae and increased the biomass of other orders (Homoptera, Diptera, Coleoptera, etc.).Two species of birds in treated sites had decreased total gut biomass. The investigators conclude that DFB has an indirect adverse effect on forest birds by reducing the availability of Lepidoptera larvae.	Sample et al. 1993a
Aerial application of Dimilin 25-W at a rate of 70.75 g/ha as part of a gypsy moth suppression program in WV.	Treatment adversely affected Lepidoptera resulting in decreased abundance and species richness; no effects were observed among Coleoptera, Diptera, or Hymenoptera. Trap catches of 3 families of Hymenoptera were unaffected, including two parasitic families, Ichneumonidae and Braconidae.	Sample et al. 1993b
Application of Dimilin *on a regular basis* (i.e., 8 applications between May 16th and December 14th 1977) to a small citrus grove in which there were two bee hives.	The hives remained in the same location throughout the study and were covered with plastic as a means of protection. There were no adverse effects on brood development of honey bees.	Schroeder 1978a MRID 00099731

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Aerial application of 350 g a.i./ha diflubenzuron to a commercial citrus grove to control *Diaprepes abbreviatus*	Residues ***in ppm*** on fruit harvested 27 days after the 6th application were: 0.34 on unwashed fruit; 0.11 on washed fruit; 0.26 on dried pulp; 0.31 on peel fruit; 0.12 on chopped peel; and 20.55 in oil.	Schroeder 1980
Additional Notes on Schroeder 1980: No detectable residue (<0.05) of DFB was found in the finisher pulp, fruit juice, pressed liquor, molasses, prewash or afterwash water, and emulsion water fractions. No detectable residue (<0.05) of 4-chlorophenylurea or 4-chloroaniline was found in the citrus fractions or in the prewash or afterwash water. The total sealed brood in honey bee (*Apis mellifera*) was not significantly different from control at 7 months and there was no detectable residue (<0.05 ppm) of DFB,CPU, or 4-chloroaniline was found in the honey obtained after 8 aerial sprays. Populations of non-target citrus pests and beneficial species were not affected by the spray program.		
Sour orange (*Citrus aurantium*) trees sprayed to runoff with Micromite 25W at 149 or 298 g a.i./1000 liters Efficacy study.	Diflubenzuron, formulated as Micromite 25W, significantly affected the reproductive potential of the sugarcane rootstock borer weevil, *Diaprepes abbreviatus* (pest of sugarcane and citrus).	Schroeder 1996
Aerial application of Dimilin formulated as 25% wettable powder at the rate of 140g/ha to 770 m square plots with a buffer strip of at least 150 m between adjacent plots in May 1985 and 1986.	Estimates of density of white-footed mouse, *Peromyscus leucopus*) did not differ significantly ($p>0.05$) between treated and untreated areas. Juvenile/adult female ratios on untreated areas were significantly higher ($p<0.05$), compared with those on treated sites. Mice on treated sites consumed less Lepidotera prey, compare with controls ($p<0.05$); however, the total amount of food consumed per mouse did not differ significantly between treated and untreated areas ($p>0.05$). There were no treatment-related adverse effects on body measurements, weight, or fat content.	Seidel and Whitmore 1995
Aerial application of Dimilin (NOS) at a rate of 140 kg/ha. The application rate is presumably a.i. but this is not specified in the publication.	No effect on bird populations that could be attributed to diflubenzuron. Various changes in the populations of different bird species are discussed but detailed data are not reported in the publication.	Stribling and Smith 1987
Simulated aerial application of diflubenzuron in acetone or in fuel oil each at 90 g a.i. in 18 L/ha to spruce foliage (*Picea glauca*).	The residue levels 1 hour after application varied, respectively, from 23.8 to 30.6 µg/g in foliage and from 3.08 to 4.60 µg/g in litter. Forty-five days after spraying the residue levels in foliage were 0.80 and 3.9 µg/g, respectively, for acetone and fuel-oil formulations.	Sundaram 1986 MRID 00161955 Sundaram 1986

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Simulated aerial spray application of technical grade DFB in acetone formulation with tracer dye or in fuel oil with tracer dye at a rate of 90 g a.i. in 18 L/ha to white spruce foliage of uneven height. The forest floor was flat and covered with grass and moss patches.	The half-lives for DFB in foliage, litter, and soil for the acetone-based formulation were 9.30, 8.36, and 7.49, respectively. 45 days after application, the residue levels in foliage were 0.80 µg/g (fresh weight) for the acetone-based formulation. There were no detectable residues in litter or soil on the 45th day post application of the acetone-based formulations.	Sundaram 1996
Soybeans in S.C. treated with 281 or 562 g a.i./ha (4 or 8 oz a.i./ac)	Significantly fewer nabids and geocorids on treated v. control sites.	Turnipseed et al. 1974
Aerial application of 8 oz Dimilin WP-25/acre (equivalent to 0.0625 lb/acre) to 10-acre mixed hardwood-conifer forested plot near Boone N. Carolina, which consisted of a stream, two stream pools, and a stream-fed pond outside the treated area. Sandy loam soil. Cumulative rainfall of 43.1 cm (16.9 inches) over a 1 year period. Daily rainfall and temperature data are given.	Initial concentration on leaves in canopy of 13 ppm on hardwood and 5.9 ppm on conifer. Initial concentrations on understory vegetation of about 0.13 ppm that increased initially as with litter. Diflubenzuron was rather persistent on leaf litter. Initial residues of 0.07 ppm. This increased over a 60 day period, probably due to drying of litter, washoff of DFB, and leaf fall from canopy.	Van Den Berg 1986 MRID 00163853

Additional Notes on Van Den Berg 1986: A single application resulted in initial water concentrations in treatment area of 0.127-0.203 ppb. Declined to 0.029-0.045 ppb after one day. No detectable contamination in an adjacent pond after heavy rains. Initial soil concentrations of 0.02 ppm and 0.03 ppm after a 6.5 cm rain (probably washoff). No DFB in 3"-6" soil samples. The study authors conclude that the effects on the mites and collembolans present at the time of application were insubstantial. In general, fewer of each group on treated than untreated sites. The data are somewhat difficult to interpret because of erratic capture patterns over time the populations of collembolans were different at the control and treated sites prior to treatment. [NOTE: Data on other species presented in Tables 10 and 11 but the numbers of insects are too small for analysis. Species list in Table 11 cut off on fiche]

Internal Note: See Van Den Berg 1986.xls may want to make figures if time.

28 g a.i./ha (0.4 oz a.i./ac)	Wasp parasite on Gypsy moth larvae (*Cotesia melanoscelus*) Pathogen: gypsy moth nuclear polyhedrosis virus (NPV). Numbers of the wasp no different on Control v. treated plots. Incidence of NPV significantly lower in treated plots. Late instar spraying may preserve larvae long enough for parasitoid to complete development. Earlier spraying kills host too quickly, hence parasitoid as well. NPV lower in treated plots because fewer Gypsy moths to transmit virus.	Webb et al. 1989

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
application of 2 or 4 oz a.i./acre [140 to 280 g/ha] to plots with large, active nests of yellowjackets	Yellowjackets (*Vespula* or *Dolichovespula*). Treatment decreased populations and the effect was readily observed during the following year. No effects observed on Mound-building ants (*Formica)*	Weiland 2000 MRID 45245403
560, 280, and 140 g a.i./ha (8, 4, and 2 oz a.i./ac). 2 and 3 treatments. Handgun and air-carrier sprayer.	Nearly twice as many *Psylla* predators and parasites per season in the lowest application rate. Higher rates resulted in higher populations of the pear psylla.	Westigard 1979
Aerial application of 0.03lbs a.i./acre Dimilin 25WP to Appalachian forest ecosystem during 1991 season (20 trees representing 7 species) in WV Univ. Experimental Forest.	Residue on leaves: significant loss of DFB from foliage ranging from 20 to 80% within the first 8 days after application; remaining DFB generally persisted for the rest of the growing season until leaf fall, at which time 13/20 treated trees retained more than 20% of the original pesticide applied.	Wimmer et al. 1993
Dimilin (TH-6040) formulated as dispersable powder (a.i. 25% by weight) applied aerially at the rate of 0.28 kg a.i./ha (0.25 lbs a.i./acre) to a Douglas-fir forest ecosystem in British Columbia	Treatment decreased the total number of flying insects and the effect was sustained throughout the study period, with the greatest impact observed on midges and gall gnats. Mosquitoes were completely wiped out as a result of treatment.	Wilson and Wan 1977a MRID 00095419 Wilson and Wan 1977b MRID 00129973 [Appear to be duplicate submissions.]

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Two applications (NOS) of granular diflubenzuron at 0.11 kg a.i./ha (about 3.7 µg/L) or 0.22 kg a.i./ha (about 7.4 µg/L) to residential-recreational lakes in San Bernadino County (June 1967 - January 1977)	**At 0.11 kg a.i./ha application**: *Daphnia pulex* and *Daphnia galeata*: 62-75% decrease in population during 7 days after treatment; populations recovered in the second week after treatment.	Ali and Mulla 1978a

Additional Notes on Ali and Mulla 1978a: 0.11 kg/ha continued. *Diaptomus* spp. (copepods): 30% decrease in population observed 2 days after treatment. *Hyalella azteca* (amphipods): 97% decrease in population observed 3 weeks after treatment; populations remained below pretreatment levels throughout 8-9 week evaluation. Treatment had no detectable effects on *Cyprihnotus* sp.(seed shrimp), *Cyclops*, or *Bosmina longirostris* (Cladocera).

At 0.22 kg a.i./ha application: *Daphnia pulex* and *Daphnia galeata*: completely eliminated for 3 weeks after treatment. *Diaptomus* spp (copepods): populations decreased to 0 within 7 days after treatment, but recovered completely *soon thereafter*. *Hyalella azteca* (amphipods): 30-100% decrease in population during 2 ½ months after treatment. *Cyprihnotus* sp.(seed shrimp): population *stressed for only 2 weeks*. *Oligochaete* (mostly *Naididae* found in marine, brackish, and freshwater habitats): no significant effects observed at either treatment level.

Two spray application of diflubenzuron (25% WP) to entire surface of residential-recreational lake in Riverside County at a rate of 156 g a.i./ha-surface (about 0.012 ppm) in April and August 1977.	**First application (April)** *Daphnia leavis* and *Ceriodaphnia* sp: population eliminated within 1 week with **no recovery** 6 months after treatment.	Ali and Mulla 1978b

Additional Notes on Ali and Mulla 1978b: *Bosmina longirostris* (cladocerans): population eliminated within 1 week with recovery after 11 weeks. *Cyclops* sp. (crustaceans): population eliminated within 1 week with recovery within 6-7 weeks. *Diaptomus* spp. (copepods): population eliminated within 1 weeks with recovery after 4 months. *Hyalella azteca* (amphipods): population eliminated within 4 weeks with **no recovery** 6 months after treatment. *Caenis* sp. [Hemeroptera (mayflies, immature)]: elimination within 3 weeks with recovery within 6-7 weeks. *Physa* sp. (sinistral snails, referred to as pond snails or pouch snails): no adverse effects. *Cypridopsis* sp.(bivalve): no adverse effects. **Second application (August)** *Bosmina longirostris* (cladocerans): population eliminated after 1 week; reappearance in small numbers 8-9 weeks after treatment. *Cyclops* sp. (crustaceans): population eliminated within 1-2 weeks with recovery after 4 weeks. *Diaptomus* spp. (copepods): population absent prior to treatment; reappearance in small numbers 1-2 months later. *Caenis* sp. [Hemeroptera (mayflies, immature)]: elimination within 2-3 weeks with recovery within 4-5 weeks.
Study does not provide monitoring data. See Ali et al. 1988 below.

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Application via airblast sprayer of Dimilin 25 WP at a rate of 0.56 kg a.i./ha to 0.8 ha of citrus immediately surrounding a pond located in Winter Garden, FL. The pond was exposed to air-drift diflubenzuron from surrounding citrus area commercially treated for the control of citrus rust mite. The control pond was located 0.4 km NE of the exposed pond.	No apparent adverse effects on zooplankton and benthic invertebrates in treated pond. Minor reductions of copepods and cladocerans during post-treatment period most likely due to short life cycle, seasonal population changes, and possible sampling deficiencies. Largest detected diflubenzuron residue = 197 ppt, 2 days after application with levels returning to trace amounts (<27 ppt) by day 14 after application. Specifics on the pond: circular,2 ha at the surface; 3/4 of its border was lined by citrus trees.	Ali et al. 1988
One surface application (via rowboat hand sprayer)of Dimilin (25% WP in 20.5 L water) to each of three ponds (0.6-0.2 hectares) at rates of 2.5, 5, or 10 ppb a.i. in California to control gnats (*Chaoborus astictopus*)and one application to a large lake at a rate of 5 ppb a.i. Surface area of ponds ranged from 0.06-0.2 ha; ponds were rectangular in shape with steep sides and flat bottoms.	Treatment was effective against gnats, decreasing larval abundance by 99%. Crustacean zooplankton populations declined precipitously at all application rates, but the effects were not permanent. Cladocerans were more susceptible than copepods and required longer recovery period. *Anabaena* sp (blue-green algae) decreased by approximately 70% within 2 weeks after treatment and remained at low levels throughout the study period; treatment seemed to have no effect on diatoms or green algae. The bioaccumulation of diflubenzuron in bluegill sunfish diminished rapidly as the residues in water decreased. No effect on growth of bluegills.	Apperson et al. 1977 MRID 00099897 Apperson et al. 1978

Additional Notes on Apperson et al. 1977, 1978: The investigators indicate that no severe or permanent nontarget effects were observed in this study. ***Residues***: In pond water, residues in the 10 and 5 ppb ponds 1 hour after treatment ranged from non-detectable to 23.6 and 32.2 ppb and averaged 9.8 and 4.6 ppb, respectively and residues levels in the 2.5 ppb pond at 4 hours after treatment ranged from N.D. to 8.3 ppm with an average of 1.9 ppb. Maximum values in bottom water samples in the 5 and 2.5 ppb ponds occurred at 4 hours and 14 days and averaged 5.3 and .5 ppb, respectively. The DFB residues declined steadily soon after treatment and at the end of the study, levels averaged 0.2, 0.3, and 0.5 ppb for the 10, 5, and 2.5 ppb ponds, respectively. No residues were found in the sediment samples.

Applications to test ponds at 1X and 4X of the typical application rate.	No effects on invertebrates or fish. [This study is poorly documented and should be given minimal weight.]	Birdsong 1965
Four applications of Dimilin W25 to ponds located in Salt Lake County Utah between 7/14/15 and 10/7/75	Algae (*Plectonema*) degraded 80% of the TH-6040 in a 1-hour incubation period. Degradation products were primarily p-chlorophenyl urea and p-chloroaniline.	Booth and Ferrell 1977 MRID 00099884

Additional Notes on Booth and Ferrell 1977: Bacteria (*Pseudomonas* sp.) accumulated “rather large amounts” of TH-6040 from the incubation media when used as the sole carbon source. No degradation products were observed in the media. Channel catfish id not bioaccumulate DFB residues from treated soil in a simulated lake ecosystem constructed in the laboratory.

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Repeated, pulsed exposures of diflubenzuron on twelve outdoor aquatic mesocosms (0.1 ha each). Random assignment of mesocosms (four/treatment) to either monthly (five total 10 µg/L applications) or biweekly (nine total 10 µg/L applications). Direct and indirect impacts on mesocosms were measured over 16 weeks after treatment.	Within 4 weeks after monthly and biweekly treatment, direct effects on Cladocerans, Copepods and Rotifers included 5-fold decrease in total numbers, 2-fold decrease in species richness, and 2-fold increase in zooplankton. Direct reductions in the numbers of invertebrate grazers caused indirect increases in algal biomass. Decreased invertebrate numbers resulted in decreases in invertebrate food resources that resulted in a 50% reduction in both biomass and individual weights of juvenile bluegills. There were no statistically significant impacts observed on adult bluegills or largemouth bass for the duration of the study.	Boyle et al. 1996

Additional Notes on Boyle et al. 1996: DFB concentrations averaged 9.9 µg/L 24 hours after chemical application. The half-life of disappearance of DFB from water, calculated across all ponds and dates using a negative exponential decay model was 2.33 days (range = 1.76-2.96 days). There were no significant differences in DFB dissipation rate between treatment type (monthly or weekly; $p \geq 0.5815$) or season (early or late in the study; $p \geq 0.4728$.

aerial application of 35 g/ha in Canada	No toxic effect on bullheads or sunfish.	Buckner et al. 1975
Two ground spray applications (at 2-week intervals) to each of two CA sites (one in Tiburon, Marin County and one in Roseville, Placer County). The first Tiburon application = 13 g/ha (0.19 oz/acre) and the second Tiburon application = 35g/ha (0.5 oz/acre);both Roseville applications = 26.25 g/ha (0.38 oz/acre) of Dimilin 25W (diflubenzuron 25% a.i.). Foliage was sprayed to the point of drip. Each site was approximately 0.8 ha. The applications were made in March-April 1990.	***Foliage***: DFB concentrations from 0 (not detected) to 18.31 µg/g immediately after the second application; and from 0(not detected) for background to 0.252 µg/cm^2 leaf area immediately after the second application. 28 days after the second application, the DFB concentration decreased sharply suggesting possible degradation during that period, but no samples were collected during the 28 days to document a degradation trend. ***Air***: During 3 of the 4 applications, DFB concentrations in air ranged from 0.0106 to 0.0187 µg/m^3. DFB was not detected in any background air samples or in any 1 day post application air samples (i.e., DFB was detected in air only during application periods). ***Water***: Samples collected from streams and water bodies in and near the treated areas on the day prior to application, immediately after each application, and 7 days after each application showed no detectable levels of DFB (minimum detection limit = 0.5 ppb).	Carr et al. 1991

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Application (NOS) of diflubenzuron to five experimental, rectangular ponds in Lakeport, CA, yielding a mean concentration of 13 µg/L DFB. Each pond had a surface area of about 0.01 ha (1 ha =10,000 m^3) and a depth of 1.2 m.	**Residues in water** decreased below detectable limits (0.2 µg/L) by 14 days after treatment; at one hour after treatment, the mean concentration of DFB in water was 13.2 µg/L.	Colwell and Schaefer 1980
Additional Notes on Colwell and Schaefer 1980: Cladocerans: most abundant species included *Ceriodaphnia*, *Diaphanosoma*, *Chydorus*, *Bosmina*, and *Daphnia*, all of which showed population reductions in all treated ponds within a few days of DFB application. **Copepods**: abundance of naupli decreased in all ponds after treatment and returned to pretreatment levels from 7 days to >4 weeks after treatment. *Diaptomus* (filter feeders) and *Cyclops* were similar in their susceptibilities to DFB, although in most of the treated ponds, *Diaptomus* populations recovered more rapidly than *Cyclops* populations. **Rotifers**: *Brachionus*, *Keratella*, and *Hexartha* populations increased in treated and control ponds during the first 8 days after treatment. *Asplanchna*, which are mostly predatory increased from 0.18 to 0.43 organisms/L after treatment. **Fish**: Young-of-the year black crappie, *Pomoxis nigromaculatus*, and brown bullhead, *Ictalurus nebulosus*, accumulated DFB and then eliminated all residues by day 7 after treatment. No fish mortalities occurred after treatment. For 1 month after treatment, the stomach content analyses of exposed fish indicated major alterations in diet. Neither growth rates or general condition of the fish 3 months after treatment differed from those of controls.		
Six aerial applications of 28 g/ha of diflubenzuron over 18 months (June 1974 through Sept 1975) to a Louisiana intermediate marsh	Treatment resulted in statistically significant differences in population density of non-target aquatic organisms (target organism - mosquito), compared with controls, but none of the affected organisms were completely eliminated from the ecosystem. The investigators speculate that the untreated marsh areas would provide populations of aquatic organisms that could repopulate the treated areas.	Farlow 1976 MRID 00099678 [Also published as Farlow et al. 1978]
Six applications of diflubenzuron (28 g a.i./ha) in a Louisiana coastal marsh over an 18-month period.	Statistically significant differences in the population density of aquatic organisms; however, none of the organisms affected were completely eliminated from the ecosystem.	Farlow et al. 1978

Additional Notes on Farlow et al. 1978: Significant populations decreases observed in five taxa: nymphs of *Trichocorixa louisianae* (water boatman) and *Buenoa* spp.(backswimmers), Coenagrionidae naiad spp.(damselflies), *Berosus infuscatus* adults (water beetles), and *Hyalella azteca* (amphipods). Significant increases were observed in populations of 15 taxa exposed to diflubenzuron, i.e., *Physa* sp. (snails), *Ceanis* sp. and *Callibaetis* sp. naiads (mayflies), *Noteridae* larvae (water beetles), *Hydrovatus cuspidatus*, adults (water beetles), *Hydrovatus* sp. larvae (water beetles), *Dytiscidae* larvae (great diving beetle), *Mesovelia mulsanti* adults (water treaders), *Trichocorixa louisiana* adults (water boatman), larvae of Chironomidae (non-biting or true midges), Ephydridae (shore flies), Dolichopodidae (long-legged flies) and Tabanidae (horseflies), as well as mosquito fish (*Gambusia affinis*) and American flag fish (*Jordanella floridae*). The 27 remaining aquatic organisms (members of the Hemiptera, Coleoptera, Mysidacea, Decapoda, Diptera and Odonata) showed no statistically significant differences, compared with untreated populations.

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Aerial application of Dimilin 4 L at a rate of 35.1 g a.i./ha to two stream catchments in the Fernow Experimental Forest, WV in May 1992.	Treatment decreased the adult emergence of stoneflies, *Peltroperla arcuata*, during the first 4 months after treatment, compared with untreated catchments. Adults populations of other species did not decrease in the treatment catchments during the period of study.	Griffith et al. 1996

Additional Notes on Griffith et al. 1996: The investigators speculate that additional detritivourous species might have shown an adverse effect if the monitoring were extended through the period after treated leaves entered the streams. Stoneflies *are considered to be obligate large-particulate organic matter feeders and like ingested diflubenzuron from leaves that fell earlier in the year, thus ingesting diflubenzuron*. Diflubenzuron was not detected in water samples taken from the streams following treatment, perhaps due rainfall just prior to treatment.

Aerial application of Dimilin 4 L at a rate of 35.1 g a.i./ha to two stream catchments in the Fernow Experimental Forest, WV in May 1992. During 1993, no additional diflubenzuron was applied to any of the watersheds.	The investigators tested the hypothesis that diflubenzuron affected adult flight following emergence during the year following abscission and possible ingestion of the treated leaves. The flight of the stonefly, *Leuctra ferruginea*, was reduced in the treatment watersheds, compared with the reference watersheds during the year following abscission of the treated leaves. Adult flight of other species did not decrease in the treatment watersheds during 1993.	Griffith et al. 2000
Aerial application of Dimlin 4L at a rate of 70 g a.i./ha to two of four watersheds in the Fernow Experimental Forest, WV.	Stream macroinvertebrate taxa that had reduced mean densities in treated watersheds ($\propto = 0.05$) included the stoneflies, *Leuctra* sp. and *Isoperla* sp., mayflies, *Paraleptophlebiaspia* sp., and cran flies, *Hexatoma* sp. Shredders, the dominant functional feeding group also had reduced mean densities in treated watersheds. Densities of Oligochaeta (aquatic worms) and Turbellaria (flat worms) increased in treated watersheds.	Hurd et al. 1996
Spray application (via portable garden sprayer) of Dimilin (25% wettable powder) at recommended rate of 0.03 lbs a.i./acre or 4X application rate to each of two 10-acre earthen ponds (avg depth of 3 ft). 4X applications were made biweekly beginning in early Feb.	No appreciable mortality of fish or clams in any of the ponds. Treatment significantly decreased *Daphnia spp*. populations and virtually eliminated dipterans. Olgochaete populations, which increased in the control pond during the study, decreased in response to treatment.	Jackson 1976 MRID 00099891

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Aerial application of 0.06 lbs a.i./acre (67.26 g/ha) Dimilin to 75-acre watershed containing small, first order stream.	Dimilin reached the stream channel during aerial application and as a result of wash-off from the foliage during several subsequent rainfall events. DFB levels (measured) exceed the acute (1.0-1.8 ppb) and chronic (60 ppt) toxicity doses for tolerant taxa, like Ephemeroptera (mayflies) and Plecoptera (stone flies). The residence time for Dimilin in these high-gradient streams was very short, and as a result of the short residence time or low concentrations, toxic effects were not evident.	Jones and Kochenderfer 1987
Spray application of 60 g a.i./ha diflubenzuron to five Sahelian temporary ponds (surface areas 0.36-0.65 ha) conducted in mid-September (half-way through rainy season) in vast savannah-type cultivated region in Senegal's ground-nut producing area. Table 1 provides a summary of wind speed, surface area treated, quantity of formulation applied in mL and calculated application rates at each of the 5 treated ponds.	Average initial concentrations in water = 10.4 µg/L, with an estimated half-life of <24 hours. DFB only affected crustaceans (i.e., cladocerans and fairy shrimp) in the treated ponds. DFB virtually eradicated the abundant fairy shrimps, *Streptocephalus* spp., and the populations did not recover despite the rapid disappearance of DFB. In general, cladocerans populations were initially wiped out (densities dropped to 0) after DFB treatment but returned to normal values in 3-4 weeks (*M micrura*), 4-6 weeks (*D senegal*), or 6-7 weeks *C quadrangula*).	Lahr et al. 2000
Application (via backpack sprayer) of Dimilin WP-25 at 280 g/ha a.i. 5 days after emergence of rice leaves out of the water to sic 20 m^2 flooded plots in June 1991 and 1992.	Field dissipation rates were similar for the six replicate plates with a half-life (1st order) of 27 hours; residues dropped to below detection limit after 96 hours. Residues in sediment were 0.16 µg/g (after 24 hours), 0.10 µg/g (after 48 hours) and 0.08 µg/g (after 72 hours); residues were below detection limit after 4 days.	Mabury and Crosby 1996
Spray application (via hand sprayer)of Dimilin 25% WP (TH6040) to semi-natural pools at the Univ. Delaware Experimental Farm to study the cumulative toxicity to killifish (3 applications over 29 days) and crustaceans (one 13-day test and one 15-day test). Applications were made at the rate of 0.01, 0.04, 0.10, and 0.20 lbs a.i./acre – i.e., up to 224 g/ha.	There was no significant mortality in killifish after three successive applications of Dimilin at 0.01-0.20 lbs a.i./acre. Behavioral responses were similar to those of controls. In the first test involving crustaceans, grass shrimp mortality was 83.3% ($p<0.01$) after the first application of 0.20 lbs a.i./acre. After two applications the average mortality ($p<0.01$) was 86.6% at 0.4 lbs a.i./acre and 100% at 0.10 and 0.20 lbs a.i./acre.	McAlonan 1975 MRID 00099895

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Additional Notes on McAlonan 1975: In the second test involving crustaceans, grass shrimp average mortality ($p<0.01$) was 91.6% at 0.4 lbs a.i./acre, 96.6% at 0.10 lbs a.i./acre, and 98.3% at 0.20 lbs a.i./acre. In the first test involving crustaceans, fiddler crab average mortality was 60.0% and 46.6% ($p<0.01$) after one application of 0.10 or 0.20 lbs a.i./acre, respectively. After two applications of 0.04 and 0.10 lbs a.i./acre the average mortality ($p<0.01$) was 53.3% and 66.6%, respectively. In the second test involving crustaceans, fiddler crab average mortality ($p<0.05$) was 46.6% at 0.4 lbs a.i./acre,60.0% at 0.10 lbs a.i./acre, and 66.6% at 0.20 lbs a.i./acre.		
Aerial application of 0.56 kg a.i./ha (8 oz a.i./acre) Dimilin 25 WP to a citrus grove in Florida with an experimental pond	DFB was not observed in water samples at quantitative methods 1 hour post application; maximum levels occurred at 1 and 2 days post application, primarily along the line of drift. Pad data indicate that the pesticide drift deposited along a small portion of the shoreline at a rate 7% of the theoretical application rate (38÷104÷5.6) and the drift continuing out into the pond was as much as 0.8% the application rate (4.4÷104÷5.6).	Nigg and Stamper 1987 MRID 40197002
Dimilin 4L at a rate of 80g/ha (0.03 lb/acre) in two forest watersheds	Decreased populations of stoneflies in treated areas. In untreated areas, the populations of stoneflies increased. After treatment, populations of roundworms, flatworms, and segmented worms were higher in treated areas.	Perry 1995a
Aerial application of 0.0624, 0.125, or 0.25 lbs/acre Dimilin to plots in Oxbow, Maine that included four streams. [up to 280 g/ha]	Effects of a single application (to control spruce budworm) on stream invertebrate fauna (*Trichloptera*, *Plecoptera*, *Ephemeroptera*, *Diptera*, *Odonata*, and *Coleptera*). No pattern of decrease in any individual genus; no treatment-related increase in drift among samples; no treatment related changes in the number of dead drift when collections were made 1-2 days after treatment.	Rabeni and Gibbs 1975 MRID 00159905
Application (NOS) of 1.25 ppm Dimilin 25WPfor 1 hour on July 13, 1984 to four points of the Kokawa River in the Izu Peninsula to control blackflies. The gradient of the river was approx. 2% and sampling stations are located between 50 and 250 m above sea level.	Most invertebrates were eliminated within 2 weeks, while Hydropsycidae (caddisfly) died out gradually. Adults of Elmidae (Riffle beetles), previously absent, appeared 1 week after treatment in large numbers at the uppermost of the treated region. No fish mortality was observed.	Satake and Yasuno 1987

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Aerial application of Dimilin WP-25 at a rate of 70 g a.i. in 10, 5, and 2.5/H to three spray blocks in a mixed boreal forest near Kaladar Ontario Canada. Water, sediment and aquatic plants were analyzed for DFB residues. Ponds appear to have been directly sprayed.	The duration of detectable DFB residues in water, sediment, and aquatic plants differed for each substrate but in all cases was less than 2 weeks. There was significant mortality in two groups of caged pond invertebrates (amphipods and corixidae [water boatman]) 1-6 days after treatment. Three taxa of littoral insects (mayflies, dragonflies, and damselflies) were significantly reduced in abundance in treated ponds 21-34 days post treatment but recovered to pre-treatment levels by the end of the season. Cladoceran and copepod populations were reduced 3 days after treatment and remained suppressed for 2-3 months.	Sundaram et al. 1991
5 monthly surface applications of 0.05 lbs a.i./acre Dimilin (25% WP) [56 g/ha] to artificial pond containing mosquito fish (*Gambusia affinis*)	No adverse effects on population growth of fish.	Takahashio and Miura 1975 MRID 00016545
2x application of Dimlin W-25 at a rate of 0.03 lbs a.i./acre at 14-day interval to an outdoor 750 gallon aquarium containing pond water and sediment, bluegill sunfish, clams, and crayfish; fate of diflubenzuron in all elements of the simulated ecosystem was monitored for 42 days from initial treatment.	Rapid dissipation of DFB (half-life < 12 hours); rapid accumulation of compound by fish and clams with rapid elimination (plateau of approx. 55 ppb by day 27 which was maintained for the duration of the experiment); fish samples contained several degradation products (CPU and DFB represent the only organo-extractable residues; clam samples contained only DFB; crayfish did not accumulate any of the compound during the week after the initial treatment.	Thompson-Hayward Chemical Co 1979 *In*: Technology Sciences Group Inc. 1998 MRID 44460702
Aerial application of Dimilin at a rate of 4.5 kg/ha (4 lbs granules/acre) to a tidal flood plain of the Fraser River in British Columbia in June 1976 . The organisms in the tidal flats of the Fraser River at the time of the study included crustaceans (zooplankton), insects, water mites and bugs, snails, and clams.	**Residue**: Dimilin, which was detected in the water up to 71 days after treatment, peaked at 1.8 ppb 8 days after application and decreased slowly to a minimum level of 0.24 ppb at 2 months after application. In mud, Dimilin peaked at 5.66 ppb 4 hours after application and decreased to a minimum level of 1.3 ppb by 2 months after treatment. **Biological effects**: Treatment arrested mosquito development but also decreased the population of zooplankton and suppressed the emergence of non-target insects of the same order as the mosquitoes.	Wan and Wilson 1977 MRID 00095416
Dimilin forestry spray at 67 g DFB/ha	No effect on aged brown trout in stream from day -7 to day +6. Observations along length of stream revealed no indication of fish mortality. Based on population estimates 6 weeks following application, no delayed effects on fish populations.	White 1975

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Broadcast foliar spray at rate of 0.25 lbs a.i./acre of Dimilin 2L to rice paddy test plots in Arkansas and California 40 days after rice planting.	DFB and its metabolites (DFBA and CPU) dissipated rapidly in the aquatic environment and there was no downward movement of DFB or its degradation products in aquatic soil/sediment.	Willard 1999 MRID 45009601
Broadcast spray application of Dimilin 25W to entire surface area of pond (containing fish) at a rate of 0.36 lbs a.i./acre.	calculated half-life for DFB in water = 5.4 days calculated half-life for DFB in soil/sediment = 8.6 days.	Willard 2000a MRID 45191001
Benthic communities in outdoor experimental streams , concentrations of 1 or 10 mg/L diflubenzuron for 30 minutes	No drift of macrobenthos was induced at the time of application. However, diflubenzuron affected the emergence of all species examined. High larval mortality for a species of chironomid was observed directly in the stream treated with diflubenzuron, where numbers of mayfly nymphs and caddisfly larvae were also decreased	Yasuno and Satake 1990

Appendix 4: Toxicity of diflubenzuron and diflubenzuron formulations to birds

Species	Nature of Exposure	Exposure Time	Effects	Reference
Single Dose				
Mallard ducks, males and females, 10 birds/dose group	single gavage doses ranging from 1000 to 5000 mg/kg bw TH-6040 (99.4% pure)	single dose	No mortality, no signs of abnormal behavior or toxicity, and no gross pathological changes to organs. NOEC = 5000 mg/kg bw	Roberts and Parke 1976 MRID 00073936
Bobwhite quail	5000 mg/kg single gavage dose	single dose	LD_{50} >5000 mg/kg bw	U.S. EPA/OPP 1997a
Note on above study: U.S. EPA/OPP 1997a attributes this study to Roberts and Parke 1976. Roberts and Parke 1976, however, only assayed mallard ducks. A review of the CBI files did not identify an acute oral study in bobwhite quail. The above entry is included in the peer review draft *but should be deleted* in the final report unless the value can be verified.				
Red-winged black birds, *Agelaius phoeniceus*, 5 or 6/dose group	single gavage dose of 1000, 2500, 3000, 4000, or 5000 mg/kg bw technical grade (99%) TH 6040; observation period of 14 days	single dose	Mortality: 1/6 at 1000 mg/kg (considered unrelated to treatment); 0/5 at 2500 mg/kg 1/6 at 3000 mg/kg following signs of piloerection, asthenia, and ataxia; 4/6 at 4000 mg/kg 5/6 at 5000 mg/kg NOEC = 2500 mg/kg bw	Alsager and Cook 1975 MRID 00038614
Acute Dietary				
Mallard ducks	in diet concentrations ≤4640 ppm technical grade TH-6040 (purity assumed to be 100%) dissolved in corn oil	8 days	NOEC =4640 ppm; no mortality and no observable signs of toxicity.	Fink and Petrocelli 1973 MRID 00038613

Appendix 4: Toxicity of diflubenzuron and diflubenzuron formulations to birds

Species	Nature of Exposure	Exposure Time	Effects	Reference
Reproduction Studies				
Mallard ducks, *Anas platyrhynchos*, young adults, 16/sex/dose group	dietary nominal concentrations of 0, 250, 500, or 1000 ppm. Based on mean body weights (about 1.25 kg) and mean food consumption (about 160 g/day), the dietary concentrations correspond to about 0, 32, 64, and 128 mg/kg bw/day.	20 weeks	No treatment-related mortality; no overt signs of toxicity; no treatment-related effects on body weight or feed consumption; no treatment-related effects of reproduction; and no treatment-related effects on body weights of hatchlings or 14-day old survivors.. At 1000 ppm, there was slight, but statistically significant decrease in mean egg shell thickness. NOEC = 500 ppm	Beavers et al. 1990a MRID 41668001
Bobwhite quail, *Colinus virginianus*, young adults, 16/sex/dose group	dietary nominal concentrations of 0, 250, 500, or 1000 ppm. Based on mean body weights (about 200 g) and mean food consumption (about 22 g/day), the dietary concentrations correspond to about 0, 27.5, 55, and 110 mg/kg bw/day.	21 weeks (1-generation)	No treatment-related mortality, overt signs of toxicity, or effects on body weight or food consumption during experimental period. At 1000 ppm, there was a marginal decrease in the number of eggs laid. NOEC (based on possible effect on egg production at 1000 ppm) =500 ppm.	Beavers et al. 1990b MRID 41668002 Beavers et al. 1990c

Appendix 4: Toxicity of diflubenzuron and diflubenzuron formulations to birds

Species	Nature of Exposure	Exposure Time	Effects	Reference
Bobwhite quail, *Colinus virginianus*, adults	dietary nominal concentrations of 2.5, 25, or 250 ppm *air-milled* (99.9% pure) diflubenzuron	12 weeks	No adverse effects on the reproductive parameters measured, including eggs laid, cracked eggs, eggs set, fertile eggs, hatched eggs, egg shell thickness, feed consumption, adult deaths, or chick survival. NOEC = 250 ppm based on review by U.S. EPA/OPP 1997a. The study authors attribute some observed differences between treated groups and controls to random variation and the large sample size (i.e, 500 eggs).	Booth et al. 1977 MRID 00099719
Chickens, White leghorn laying hens, 27-weeks old 10/dose group	dietary nominal concentrations of 0, 10, 50, 100, or 500 ppm diflubenzuron	8 weeks	No adverse effects on food consumption, body weight, egg production, egg weight, egg shell thickness, fertility, hatchability, or progeny development. Diflubenzuron accumulated in eggs and body tissues; 5 weeks after treatment, diflubenzuron was not delectable in the egg, liver, fat, or muscle tissues of hens fed any of the dose levels of the compound.	Cecil et al. 1981 MRID 00156781 Cecil et al. 1981 [published in the open literature]
Growing male broiler and layer chickens	Diflubenzuron at dietary concentrations of up to 250 mg/kg feed	from 1 day of age to 98 days	No consistent differences over time on body weight, food consumption, or testes, liver, comb and feet weights.	Kubena 1981
Layer-breed chickens, males and females	diflubenzuron was fed at levels of 0, 2.5, 25 and 250 mg/kg feed	from 1 day of age through a laying cycle	No effects on egg production, egg weight, eggshell weight, fertility, hatchability or progeny.	Kubena 1982

NOS = Not otherwise specified.

Appendix 5: Toxicity of diflubenzuron to terrestrial invertebrates

Species	Exposure	Effects	Reference
House fly(*Musca domestica*) and parasitoid *Muscidifurax raptor*	Dimilin, topical exposure	No effect to eggs or pupae at 10,000 ppm. > 90% mortality to intermediate to late stage larvae at 1.25 to 10 ppm. No effects to parasitoid.	Ables et al. 1975
Gypsy moth predators: lacewing (*Chrysopa carnea*), ladybird beetle (*Hippodamia convergens*), Wasp parasite *Trichograma pretiosum* of bollworm (*Heliothis*)	10 mg on 9-cm filter paper (contact); and 5 ppm sugar-water fed to host.	Lab rearing of hosts on diflubenzuron diets and raising parasites on those eggs. And raised lacewings from topically treated eggs and adults. Negative effects on lacewing and ladybird beetle in lab; egg hatch of beetle returned to normal after 30-40 d.	Ables et al. 1977
Honey bees, *Apis mellifera L.*	Dietary exposure at concentrations of 0.59, 5.9, and 59 mg/kg diet for 10 days. Vehicle: Sugar syrup.	Reduced brood production at the highest concentration. No effect at two lower concentrations.	Barker and Taber 1977
Honey bees, *Apis mellifera L.*	Diflubenzuron (25% WP) formulation (100 ppm a.i.) supplied in water and 60 ppm supplied in sucrose syrup to colonies of honey bees in outdoor cages.	Brood production almost eliminated; treated bees consumed significantly less water and pollen cake and produced significantly less comb, brood, and new workers. Number of eggs increased in treated colonies. No significant differences in survival of treated bees, compared with controls and both treated and untreated colonies built queen cells when the original queen was removed.	Barker and Waller 1978
Rice swarming caterpillar adult *Spodoptera mauritania*	Dimilin 25-WP, dietary exposure	60-64% sterility at 10 ppm, 100% sterility at 100-1,000 ppm	Beevi and Dale1984
Gypsy moth *Lymantria dispar*	topical exposure	LD_{50} = 3.58 mg/kg (alder) LD_{50} = 8.96 mg/kg (douglas fir)	Berry et al. 1993
Gypsy moth *Lymantria dispar*	acute oral exposure	LC_{50} = 0.06 ppm diet (alder) LC_{50} = 0.45 ppm diet (douglas fir)	Berry et al. 1993
earthworm (Eisenia fetida)	soil exposure	NOEC = 1 g Dimilin WP-25 per kg dry soil	Berends and Thus 1992
earthworm (Eisenia fetida)	soil exposure	NOEC = 780 mg diflubenzuron per kg dry soil	Berends et al. 1992

Appendix 5: Toxicity of diflubenzuron to terrestrial invertebrates

Species	Exposure	Effects	Reference
Nontarget insects (lacewing *Chrysopa oculata*, braconid wasp *Macrocentrus ancylivorous*, assassin bug *Acholla multispinosa*)	Dimilin 25-WP - topical exposure up to 300 ppm and contact with treated leaves	Considerable mortality and inhibition of molting to lacewing, but no effects to wasp or bug.	Broadbent and Pree1984a
	consumption of treated host larvae	reduced emergence of wasp, but no effect on lacewing.	
cockchafer *Melolontha melolontha*, leaf beetle *Gastroidea viridula*	beech or sorrel leaves treated with 0.1% Dimilin 25-WP	repellant effects and 100% ovicidal effect to chafer. Effective against larvae and eggs of beetle.	Büchi and Jossi1979
Honey bee	Dimilin - topical exposure	LD_{50} = 52.9 mg/kg (3rd instar) LD_{50} = 45.51 mg/kg (4th instar) LD_{50} = 22.33 mg/kg (pupa)	Chandel and Gupta1992
Bee *Apis cerana indica*	Dimilin - topical exposure	LD_{50} = 56.15 mg/kg (3rd instar) LD_{50} = 49.13 mg/kg (4th instar) LD_{50} = 22.69 mg/kg (pupa)	Chandel and Gupta1992
Spined soldier bug, *Podisus maculiventris*, (predator)	Topical, residual, and oral exposure to diflubenzuron 48% suspension concentrate.	Diflubenzuron harmless to predatory bug by direct and residual contact, but highly toxic when ingested via drinking water. Five days after adult emergence, LC_{50} (for ingestion to 5th instar nymphs) = 7.20 µg/mL. Exposure of 5th instars to sublethal concentrations (around LC_{10}) had no adverse effects on reproduction of emerging adults.	De Clercq et al. 1995b
Flower bug, *Orius laevigatus*, predatory bug used as a biological control for thrips. N= 20	5th instar nymphs were exposed to formulated diflubenzuron WP 25 via ingestion of contaminated (saturated) cotton wool plug and residual contact for 3 days.	LC_{50} (residual contact) = 391.1 mg a.i./L (95% CI = 140.5-825.6 mg a.i./L) LC_{50} (ingestion) = 229.9 mg a.i./L (95% CI = 108.0-397.3 mg a.i./L)	Delbeke et al. 1997
Migratory grasshopper *Melanoplus sanguinipes*	Dimilin 25-WP, dietary exposure	LC_{50} = 0.08 ppm (lettuce diet) LC_{50} = 0.1 ppm (wheat seedling diet)	Elliott and Iyer1982
Honey bee	Dimilin - topical or dietary exposure	LD_{50} > 30 µg/bee (topical) LD_{50} > 200 µg Dimilin WP-25 per bee (dietary). No adverse effects at 5.9 ppm.	Gijswijt1978

Appendix 5: Toxicity of diflubenzuron to terrestrial invertebrates

Species	Exposure	Effects	Reference
Rove beetle (*Aleochara bilineata)* and Cabbage maggot (target)	Consumption of cabbage maggot treated with Dimilin 25-WP	No adverse effects on rove beetle. Suppression of egg hatching and larva development of the cabbage maggot *Delia radicum*	Gordon and Cornect1986
Desert locust (*Schistocerca gregaria*)	Dietary exposure	LD_{50} = 886.7 µg AI (2nd instar) LD_{50} = 207.4 µg AI (4th instar) LD_{50} = 325.2 µg AI (5th instar)	Jepson and Yemane1991
Mealworms, *Tenebrio molitor*, adults	10 mg/g technical Diflubenzuron incorporated into the diet (wheat flour) for period of ecysis to 9 days	Treatment quantitatively and qualitatively altered the lipid metabolism during sexual maturation. Fatty acid composition of the ovaries was not affected.	Khebbeb et al. 1997
Gram pod borer, *Helicoverpa armigera* (Lepidotera: Noctuidae) [crop pest] eggs 0-24 and 24-48 hours.	eggs dipped for two minutes in different concentrations (NOS) of a suspension of diflubenzuron in distilled water.	IC_{50} (0-24 hours) = 0.0055 ppm (fiducial limits= 0.007-0.004 ppm) IC_{50} (24-48 hours) = 0.0061 ppm (fiducial limits= 0.01-0.0034 ppm)	Kumar et al. 1994
Honey bee	acute topical exposure	LD_{50} > 100 µg/bee (adult) LD_{50} >0.0125 µg/bee (larva)	Kuijpers1989
Honey bee	acute oral exposure	LD_{50} > 100 µg/bee (adult) LD_{50} > 0.030 µg/bee (larva)	Kuijpers1989
Oxya japonica (Orthoptera)	Dimilin 25-WP, topical exposure	LD_{50} = 0.06 µg per insect or 0.31 mg/kg	Lim and Lee1982
Australian ladybird beetle, *Cryptolaemus montrouzierei* , adults (excellent predator of mealybug species)	200 ppm diflubenzuron on treated surface	No adverse effects on longevity or feeding; however treatment had effects on adult females, yielding only 278 progeny, compared with 419 yielded by controls.	Mani et al. 1997
Gypsy moth	Dimilin 25-WP, dietary exposure at 0.1 mg/kg	100% lethal to larvae	Martinat et al. 1988

Appendix 5: Toxicity of diflubenzuron to terrestrial invertebrates

Species	Exposure	Effects	Reference
Grasshopper, *Poekilocerus pictus*, 2-day-old, virgin females	20 µg/insect Diflubenzuron dissolved in acetone applied on the ventral side of the abdomen.	In few treated females, the abdomen could not come out of the sand after egg laying and mortality occurred in the same position. When the abdomen was stretched back, the normal position was not attained again, which may be attributed to the chitin synthesis inhibiting activity of diflubenzuron. Ovaries of treated females were adversely affected by treatment, which probably accounts for the decrease in reproduction.	Mathur 1998
Mexican bean beetle	Dimilin 25-WP, dietary exposure	LC_{50} = 3.4 ppm (3rd instar)	McWhorter and Shepard 1977
Lacewing, *Chysoperla carnea*, adults <24 hours old	topical application	At a diflubenzuron at dose of 7,000 ng/insect, no mortality among adults; 100% inhibition of egg hatching due to death embryo. At the lowest dose, 75 ng/insect), 32% reduction in egg hatch.	Medina et al. 2002
Lacewing, *Chysoperla carnea*, adults <24 hours old	topical application	LD_{50} = 2.26 ng/insect LD_{10} = 0.74 ng/insect LD_{90} = 6.87 ng/insect No effect on reproduction at a dose of 0.5 ng/insect.	Medina et al. 2003
Honey bees, caged colonies	10 mg/kg diflubenzuron for 10 weeks	No adverse effects on pollen consumption or brood production; however treatment resulted in a 50% decrease in the amount of syrup stored.	Nation et al. 1986
Cotton leafworm *Spodoptera littoralis*	Dietary exposure	LC_{50} = 1 mg/kg	Neumann and Guyer1987
Predacious phytoseiid mite, *Amblyseius womersleyi*, adult females	Diflubenzuron (Dimilin) (25% pure) at field rate of 100 ppm on bean leaf disks dipped in test substance	No mortality 3 days after treatment.	Park et al. 1996
Oncopeltus fasciatus, Large milkweed bug	Topical exposure to 1 µg/insect	Inhibition of reproduction	Redfern et al. 1980

Appendix 5: Toxicity of diflubenzuron to terrestrial invertebrates

Species	Exposure	Effects	Reference
Brown lacewing, *Micromus tasmaniae* (beneficial predator)	contact exposure: 0.07 µg/cm^2 a.i. as Dimilin 25 WP sprayed on petri dishes	Treatment caused a strong trend toward decrease in fertility where 13% of all pairs did not lay any eggs; total numbers of eggs produced per females were reduced by approx. 50%; treated females deposited significantly fewer eggs per day than the control females ($p<0.01$).	Rumpf et al. 1998
Brown lacewing, *Micromus tasmaniae* (beneficial predator)	contact exposure: Dimilin 25 WP sprayed on petri dishes 32 hours after the 2nd larval molt	120 hour LC_{50} = 0.069% a.i. (95% CI: = 0.049-0.107% a.i.) 360 hour LC_{50} = 0.009% a.i. (95% CI: = 0.003-0.012% a.i.)	Rumpf et al. 1997
European earwig *Forficularia auricularia*	12.5 g a.i./ha	growth and mobility adversely affected	Sauphanor et al. 1993
Pieris brassicae (Large White Butterfly)	Topical exposure	LD_{50} = 2.5 µg/insect or 1.07 mg/kg	Sinha et al. 1990
Mealworms, *Tenebrio molitor*, adults	5 or 10 mg/g Diflubenzuron (NOS) incorporated into diet for 3 or 6 days post emergence.	Diflubenzuron had no significant effect on fat body protein.	Soltani-Mazouni and Soltani 1995a
Mealworms, *Tenebrio molitor*, adults	5 or 10 mg/g Diflubenzuron (NOS) incorporated into diet . Duration of exposure not clear.	treatment caused a decrease in both the cell density of germarium and the thickness of chorion.	Soltani and Soltani-Mazouni 1997
Mealybug ladybird beetle, *Crptolaemus montrouzieri*, predator of mealybugs	freshly emerged final instar nymphs were fed with mealy bugs treated with 0.153 ppm Diflubenzuron and sacrificed after 24, 48, 72, or 96 hours.	There was a significant reduction in protein content after 2 hours; however, with prolonged exposure, the insect was found to adapt itself to the toxic stress and the adverse effect was much less pronounced after 96 hours.	Sundari et al. 1998
Honey bee	oral and contact LD_{50} values	>30 µg/bee	Stevenson 1978

Appendix 5: Toxicity of diflubenzuron to terrestrial invertebrates

Species	Exposure	Effects	Reference
Honey bee, *Apis mellifera*	0.1, 1, & 10 ppm in Sugar-cake for 12 wks. 0.01, 0.1, & 1.0 ppm in sucrose syrup next year for 10 weeks.	At 10 ppm diflubenzuron in sugar-cake, significantly fewer sealed brood were produced, and colony size was reduced significantly compared to control and lower dosed colonies. No effects on brood production, colony size or adult bee mortality were seen the following year, when lower doses in a fluid solution was used. Degradation in sucrose solution might have reduced the potential for adverse effects.	Stoner and Wilson 1982
Fruit-sucking moth, *Othreis materna*, 5th instar larvae	topical application of 0 or 0.025 µL Dimilin (25 WP) in 5 µL acetone to ventral region of the abdomen. Larvae were sacrificed 24, 48, or 72 hours after exposure.	Inhibition of molting in larvae seems to occur due to neuroendocrine failure. See Section 4.1.2.3. for discussion.	Tembhare and Shinde 1998
Honey bee colonies	Diflubenzuron diluted with sucrose to a rate equivalent to maximum application rate on flowering crops.	Treatment with diflubenzuron resulted in short-term decrease in the numbers of adult bees and brood, compared with controls. No significant effect on development of brood during the following spring; however, there appeared to be a slower expansion, compared with controls. No adverse effects on queen viability.	Thompson and Wilkins 2003
Nematodes	10 day dietary exposure to Dimilin at 10 ppm	Adults unaffected but reproduction hindered and egg hatch prevented. Population reductions of 5% for *Pelodera* sp., 47% for *Panagrellus redivivus*, and 94% for *Acrobeloides* sp.	Veech 1978
German cockroach *Blatella germanica*	Dimilin 25W® - contact with spray of treated cage plywood panels	population reduction of 67.3% at 30 mg/m^2, 93% at 60 mg/m^2, and 98.2% at 120 mg/m^2. egg hatch unaffected, but high first instar mortality.	Wadleigh et al.1991
Codling moth (*Cydia pomonella*), neonates of field-collected and laboratory strains	Dimilin WP	5-day LC_{50} = 13.9 mg/L (95% CI = 10.7-18.2 mg/L)	Weiland 2000 MRID 45245403

Appendix 5: Toxicity of diflubenzuron to terrestrial invertebrates

Species	Exposure	Effects	Reference
Honey bee	Dimilin 25-WP, dietary	LC_{50} = 3.7 ppm	Wittmann1982
Honey bee	Diflubenzuron dietary	No toxicity at concentrations up to 1000 mg/kg in the diet.	Yu et al. 1984
Stinkbug, *Podisus nigrispinus*, eggs and nymphs	Diflubenzuron sprayed on eggs and nymphs.	No effect on egg viability.	Zacarias et al. 1998
Host: Mexican bean beetle (*Epilachna varivestis*). Parasite: wasp (*Pediobius foveolatus*).	100, 1,000, and 10,000 ppm	Topical application to adults did not affect survival or reproduction, nor that of their progeny. Emergence of parasite from larvae treated after parasitism and before was 0 or nearly 0.	Zungoli et al. 1983

Appendix 6: Toxicity of diflubenzuron to fish

Species	Nature of Exposure	Exposure Time	Effects[a]	Reference
		Diflubenzuron		
Acute				
Bluegill sunfish, *Lepomis macrochirus*	static renewal bioassay	96 hours	LC_{50} = 135 mg/L	Marshall and Hieb 1973 MRID 00056150
Fathead minnow	static	96 hours	LC_{50} > 500 mg/L	Reiner and Parke 1975 MRID 00060376
Mummichog, *Fundulus heteroclitus*	static renewal bioassay	96 hours	NOEC = 29.86 mg/L LC_{50} = 32.99 (CL = 29.01-37.52 mg/L)	Lee and Scott 1989
Rainbow trout, *Salmo gairdneri*	static renewal bioassay	96 hours	LC_{50} = 140 mg/L	Marshall and Hieb 1973 MRID 00056150
Rainbow trout, Channel Catfish, and Bluegills	static	96 hours	LC_{50} > 100 mg/L	Johnson and Finley 1980
Brook trout	static	96 hours	LC_{50} > 50 mg/L	Johnson and Finley 1980
Yellow perch	static	96 hours	LC_{50} = 25 mg/L	Johnson and Finley 1980
Rainbow trout	static	96 hours	LC_{50} = 240 mg/L as Dimilin 25-WP	Julin and Sanders 1978
Channel catfish	static	96 hours	LC_{50} = 370 mg/L as Dimilin 25-WP	Julin and Sanders 1978
Fathead minnow	static	96 hours	LC_{50} = 430 mg/L as Dimilin 25-WP	Julin and Sanders 1978
Bluegill sunfish	static	96 hours	LC_{50} = 660 mg/L as Dimilin 25-WP	Julin and Sanders 1978
Yellow perch	static	96 hours	LC_{50} > 50 mg/L	Mayer and Ellersieck, 1986
Brook trout	static	96 hours	LC_{50} > 50 mg/L	Mayer and Ellersieck, 1986
Cutthroat trout	static	96 hours	LC_{50} > 60 mg/L	Mayer and Ellersieck, 1986
Atlantic salmon	static	96 hours	LC_{50} > 50 mg/L	Mayer and Ellersieck, 1986

Appendix 6: Toxicity of diflubenzuron to fish

Species	Nature of Exposure	Exposure Time	Effects[a]	Reference
Longer Term				
Fathead minnows	continuous exposure to concentrations of 0, 0.00625, 0.0125, 0.025, 0.05, or 0.10 ppm 99.4% pure TH-6040 (air milled)	10 months	No effects on survival, growth, behavior or reproduction, compared with controls; no observable effects on hatchability of eggs spawned by fish. Fry, hatched from eggs spawned by treated fish showed no appreciable differences, compared with controls after 60 days of exposure to TH-6040, under same conditions as parental fish.	Cannon and Krize 1976 MRID 00099755
Salmonids (steelhead trout) and non-salmonids (fathead minnows and guppies) fish species	Diflubenzuron under flow-through conditions at concentrations up to 45 µg/L.	96 hours or 30 days (survival and growth in early life stages	No effects at any concentration. NOEC >45 µg/L (highest concentration tested)	Hansen and Garton 1982a
Mummichug, *Fundulus heteroclitus* (marine species)	Life cycle involving continuous (flow through) exposure to TH-6040 dissolved in acetone to deliver concentrations of 0.003, 0.006, 0.0125, 0.025, or 0.05 ppm	life cycle (2-generations)	No significant dose-response relationships.	Livingston and Koenig 1977 MRID 014402120 Livingston and Koenig 1977 MRID 00099722
Mesocosm				
Bluegill sunfish, *Lepomis macrochirus*, "young-of-the year"	Dimilin at nominal treatment levels of 0.7, 2.5, 7.0, or 30 µg/L to littoral enclosures	70 days	NOEC = 0.7 µg/L LOEC = 2.5 µg/L Secondary effects on endpoints based on growth (individual fish size). See additional notes below.	Moffett and Tanner 1995 ***In***: Moffett 1995 MRID 44386201

Appendix 6: Toxicity of diflubenzuron to fish

Species	Nature of Exposure	Exposure Time	Effects[a]	Reference
Additional Notes on Moffett and Tanner 1995: In indigenous fish species, mean fish size, population numbers, and biomass were not affected by exposure to diflubenzuron (≤30 µg/L). Indigenous species included brook stickleback, northern redbelly dace, and central mudminnows. Young-of-the-year bluegill growth rates were directly correlated to the density of several invertebrates (cladoceran and copepods) in the enclosures and inversely correlated to the measured concentration of diflubenzuron. The results indicate that the indirect effects of diflubenzuron on bluegill sunfish were caused by a reduction in food resources due to the direct toxicity of the pesticide on the chitinous invertebrates preferred by the bluegill.				
Bluegill sunfish, *Lepomis macrochirus*	Dimilin 25 W in littoral enclosures at nominal concentrations of 2.5 or 30 µg/L	reproductive cycle	Treatment adversely affected reproductive success by decreasing growth of young of the year bluegills at 2.5 and 3.0 µg/L by eliminating or reducing preferred bluegill food choices (cladocerans and copepods).	Tanner and Moffett 1995 ***In***: Moffett 1995 MRID 44386201
Additional Notes on Tanner and Moffett 1995: No behavioral effects related to reproduction of adult bluegills were observed in the enclosures. There was no clearly determined effect on spawning; however it appeared by spawning was influenced more by water temperature than by diflubenzuron. No direct effects on larvae prior to swim-up; however secondary effects on growth were evident following swim-up, apparently due to the precipitous decrease of zooplankton and the decline of chironomids and other macroinvertebrates.				
Bioconcentration				
Bluegill sunfish, *Lepomis macrochirus*	dynamic 42-day study to evaluate bioconcentration of C ^{14}-diflubenzuron	28 days under flow-through conditions, with 14 day depuration period	In fillet, the BCF was 120 after 1 day and 170 after 28 days with a peak of 200 after 7 days. In whole fish, the BCF was 260 after 1 day and 350 after 28 days with a peak of 360 after 7 days.	Burgess 1989 MRID 42258401
White crappies	10 ppb DFB	24 hours	BCF = 82.2	Schaefer et al. 1979
Bluegill sunfish	10 ppb DFB	24 hours	Residues of approximately 848 ppb; 218 ppb in skin and 232 ppb in inner tissues (NOS); residues decreased rapidly when fish were transferred to the rinse tank for ≥48 hours.	Schaefer et al. 1979

Appendix 6: Toxicity of diflubenzuron to fish

Species	Nature of Exposure	Exposure Time	Effects[a]	Reference
		p-Chloroaniline		
Acute				
Bluegill *Lepomis macrochirus*	Static	96 hour	LC_{50} value = 2.4 mg/L	WHO 2003
Longer Term				
Medaka, *Oryzias latipes*	Larval growth; flow-through	28 days	MATC <2.25 mg/L	WHO 2003
Zebra fish *Brachydanio rerio*	growth and reproduction at 0.04, 0.2, and 1 mg/liter	5 weeks	Adverse effects at 1 mg/L: abdominal swelling, spinal deformations, reduced number of eggs, and reduced fertilization in the F1 and F2 generations.	Bresch et al. 1990
Zebra fish *Brachydanio rerio*	Flow-through	3 weeks	NOEC for Mortality and other effects = 1.8 mg/L	WHO 2003
Bioconcentration				
Medaka, *Oryzias latipes* (Killifish)	Static aqueous exposures to [^{14}**C]-chloroaniline** (8.9-17 mCi/ mmol; >98% pure) for up to 320 minutes	up to 320 minutes	Due to low elimination rates, 20% of the absorbed dose remained within the fish through 330 minutes after exposure. N-acetylation was the dominant route of *in vivo* metabolism, with no indication of ring hydroxylation.	Bradbury et al. 1993
Carp, *Cyprinus carpio*	continuous flow-through exposure to 0.30±0.07 or 10.4±0.4 µg/L **p-chloroaniline**	up to 335 hours (about 14 days)	average BCF in whole body were 1.7 (low concentration) and 0.8 (high concentration).	Tsuda et al. 1993

[a] Values in parentheses are the 95% confidence limits.

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Grass shrimp, *Palaemonetes pugio*	Subchronic exposure to measured concentrations of 0.70, 1.73, 5.51, 6.79, or 16.4 µg/L for 35 days in flowing seawater	No survival to day 7 among zoea exposed to initial measured concentrations of 5.5, 6.8, or 16.4 µg/L; survival among shrimp exposed to 0.70 or 1.73 µg/L was significantly less than survival among controls; no significant difference in size of shrimp exposed to 0.70 or 1.73 µg/L, compared with controls.	Bionomics-EG&G 1975 MRID 00038612
Grass shrimp, *Palaemonetes pugio*	Acute exposure to nominal concentrations of ≤1.0 mg/L TH-6040 in static seawater	96-hour LC_{50} = 0.64 mg/L (0.13-3.1 mg/L)	Bionomics-EG&G 1975 MRID 00038612
Hydropsychidae (Trichoptera)	Dimilin 25-WP, 15 days at 0.0025 to 0.25 mg/L	No adult emergence from treated tanks and only 31.6% emergence from control tanks	Bradt and Williams 1990
Mysid shrimp, *Mysidopsis bahia*, F_1 second generation	mean measured concentration of 123 ng/L (0.123 µg/L) diflubenzuron (97.6% pure) for up to 5 days	upon removal of treated water, juvenile second generation mysids completely recovered and had survival and reproductive success similar to that of the controls.	Breteler 1987 MRID 40237501
Mysid shrimp,*Mysidopsis bahia*, juvenile	Continuous exposure to mean measured concentrations of 29, 45, 86, 140 or 210 ng/L diflubenzuron through entire life cycle over a 28-day test period. Juvenile mysids produced during the test at the lowest four test concentrations (29-140 ng/L) were continuously exposed for the 8 days of the 28-day test.	F_0 survival at 86, 140, and 210 ng/L was significantly reduced ($p \leq 0.05$) compared with controls; treatment caused significant reduction in growth and development (as measured by dry weight) in F_0 males (210 ng/L) and F_0 females (140 and 210 ng/L); reproduction of F_0 mysids was significantly reduced at 86, 140, and 210 ng/L. The NOEC = 86 ng/L for growth LOEC = 140 ng/L for growth. Survival of the second generation (F_1) mysids was not affected by continuous exposure to any of the mean measured concentrations tested (21, 33, 83, or 123 ng/L). The NOEC after 8 days of exposure of F_1 generation mysids was >83 ng/L.	Breteler 1987 MRID 40237501 Note: This summary is of the ***primary study*** on which the studies discussed below are based.

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Mysid shrimp, *Mysidopsis bahia*	24-hour exposure to mean concentration of 298 ng/L diflubenzuron (97.6% pure), followed by transfer to clean control water for 27 days.	Survival, growth, and reproductive success similar to that of controls.	Breteler 1987 MRID 40237501
Marine crabs, *Pontonia pinnophylax*, larvae	≤10 ppb diflubenzuron	larvae of four different crab species appeared normal during inter-molt periods and adverse effects were apparent until molting (similar to effect of DFB on insect larvae). Treatment deformed both the exocuticle and the endoculticle and was lethal to all four species of marine crabs.	Christiansen 1987
Mixed aquatic invertebrates (i.e., cladocerans, rotifers, and adult amphipods)	Microcosm 1: nominal concentrations of 0.3, 0.7, 1.4, 3.4, 6.8, or 13.6 µg/L Dimilin 25W Microcosm 2: nominal concentrations of 1.4. 3.4. 6.8, or 20.0 µg/L Dimilin 25W	Major effect of diflubenzuron in the microcosms was on the cladocerans. Population density was decreased within 3-4 days after treatment at ≥0.7 µg/L and remained consistently low, compared with controls throughout the study duration. Statistically significant ($p \leq 0.05$) differences in population density at ≥1.4 µg/L in Microcosm 1 between days 3 and 10 and at ≥0.7 µg/L in Microcosm 2 between days 4 and 14. Cladoceran population densities did not generally increase in either microcosm at ≥0.7 µg/L. Rotifers were no adversely affected by treatment at any concentration. The numbers of adult amphiphods (*Hyalella azteca*) were significantly different from controls ($p \leq 0.05$) at 13.6 µg/L (Microcosm 1) and 20 µg/L (Microcosm 2). *Amphipods exposed to concentrations <13.6 µg/L were not different ($p \leq 0.05$) from controls in either experiment.* NOEC for cladocerans = 0.3 µg/L LOEC for cladocerans = 0.7 µg/L	Corry et al. 1995 ***In***: Moffett 1995 MRID 44386201

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Fiddler crabs, *Uca pugilator*, juveniles	repetitive 24-hours weekly exposures to 0.2, 2, 20, or 200 µg/L Dimilin in static seawater systems for 10 weeks.	NOEC (time to first molt) =20µg/L NOEC (survival) = 2 µg/L NOEC (ability to escape from test container) = 0.2 µg/L Behavioral effect caused by DFB exposure (≥2 µg/L) was most sensitive indicator of DFB toxicity. Investigators conclude that survival, molting, and behavior of juvenile fiddler crabs are significantly affected by exposure to repetitive applications of DFB.	Cunningham and Meyers 1987
Barnacles, *Balanus eburneus*, Cirripede crustaceans.	Exposure to1-1000 µg/L technical grade, air-milled diflubenzuron w/acetone as carrier solvent (preliminary studies showed no mortality in acetone controls) for 28 days	Dose-dependent mortality, with drastic mortality observed during the second week of exposure. Lethal and sublethal effects were observed at concentrations as low as 50 µg/L Disruption of the exoskeleton caused by diflubenzuron was similar to that observed in insects. Development of barnacles exposed to diflubenzuron for 10 days or more at 750 and 1000 µg/L was delayed in the pre-molt phase of cuticle secretion	Gulka et al. 1980
Ceriodaphnia dubia, neonates, <12 hours old	Exposure to 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, or 4.0 ng/mL Dimilin for 48 hours.	48-hr NOEC = 0.75 ng/mL [0.75 µg/L] 48-hr LC_{50} =1.7 ng/mL (95% CI = 1.36-2.02 ng/mL) [1.7 µg/L]	Hall 1986 MRID 40130601
Ceriodaphnia dubia	Chronic exposure to 0, 0.05, 0.1, 0.25, 0.5, 0.75, or 1.0 ng/mL (µg/L). Used methanol carrier with carrier control.	NOEC = 0.25µg/L At ≥0.5 µg/L, significant decrease in numbers of neonates produced, compared with controls; at 0.75 and 1.0 µg/L, adults produced no viable young; mortality increased at exposures to >0.1 µg/L. No carrier effect: 31.7 (28.4-34.9) neonates/female with 20% mortality in adults in untreated control and 30.9 (26.9-35) in carrier control with 10% mortality in adults.	Hall 1986 MRID 40130601

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
CRITICAL NOTE on HALL 1986: Hall (1986) reports concentrations as nanograms/mL. These are converted above to µg/L.			
Daphnia magna	Diflubenzuron under static conditions for 48 hours	LC_{50} = 1.84 µg/L (95% CI = 0.05-3.71 µg/L)	Hansen and Garton 1982a
Midges, *Tanytarsus dissimilis*, 2nd to 3rd larval instar	Diflubenzuron under flow-through conditions for 5 days; effect criteria = molting success	LC_{50} = 1.02 µg/L (95% CI = 0.56-1.47 µg/L)	Hansen and Garton 1982a
Midges, *Cricotopus*, sp, 4th larval instar to pupae	Diflubenzuron under flow-through conditions for 7 days; effect criteria = molting success	LC_{50} = 1.79 µg/L (95% CI = 1.48-2.13 µg/L)	Hansen and Garton 1982a
Daphnia magna	Survival and reproduction in full life cycle after exposure to diflubenzuron (conditions not specified)	LC_{50} = 0.062 µg/L (95% CI = 0.051-0.071 µg/L)	Hansen and Garton 1982a
Freshwater molluscs (two species of snails)	Diflubenzuron under flow-through conditions for 96 hours; effect criteria for chronic exposure (3 weeks) = survival, growth and reproduction	NOEC 45 µg/L (highest concentration tested)	Hansen and Garton 1982a
Stream invertebrates (most abundant), including Ephemeroptera, Plecoptera, Diptera, Tricoptera, and Coleoptera.	Technical diflubenzuron in dimethlformamide at 0.1, 1, 10, and 50 µg/L added continuously to complex laboratory stream channels supplied periodically with field-collected microorganisms for 5 months	Invertebrates were most adversely affected undergoing rapid and permanent reductions in biomass and diversity at diflubenzuron concentrations of ≥1.0 µg/L. These effects were the results of major in reductions in many of the aquatic insect populations, primarily among mayflies, stoneflies and diptera.	Hansen and Garton 1982a

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Additional Notes on Hansen and Garton 1982a: Diversity in all groups of stream invertebrates was clearly dose-related with little or no reductions observed at 0.1 µg/L, intermediate reductions observed at 1.0 µg/L (some dipteran tax were relatively insensitive at this concentration but eliminated at higher concentrations), and maximal reductions observed at ≥10.0 µg/L. Algal, fungal, and bacterial functional groups were also adversely affected by exposure to diflubenzuron. Generally the adverse effects observed among these organisms was variable and transient alterations in biomass and diversity with algae and bacteria affected at 1.0 µg/L and fungi affected at as little as 0.1 µg/L.			
Total biological community in 8 stream microcosms	8- month continuous exposure to 0.1, 1.0, 10, or 50 µg/L diflubenzuron dissolved in dimethylformamide	Insects were directly affected at ≥1.0 µg/L (stoneflies and mayflies were the most sensitive with adverse effects apparent at 1.0 µg/L, dipterans affected at 10.0 µg/L, and coelopterans were not affected at any test concentrations); Algae and fungi were mildly affected at ≥1.0 µg/L, but the effects were considered indirect in response to the decreases in herbivore and shredder components of the insects; No effects were observed in bacteria, oligochaetes, or gastropods at any test concentration.	Hansen and Garton 1982b
Gammarid, *Hyallela azteca* (Benthic crustacea)	Diflubenzuron under flow-through conditions for 96 hours	LC_{50} = 1.84 µg/L (95% CI = 0.05-3.71 µg/L)	Hansen and Garton 1982a
Stoneflies, *Peltoperla arcuata* and *Pteronarcys proteus*	DFB-treated yellow poplar leaves via ingestion for 24-hours with 60- and 90-day observation periods.	*Peltoperla*: survival significantly different from controls at day 60; however survival of *Pteronarcys* was not significantly different from controls at 90 days, although the low number of molts that occurred during that time may have influenced the results.	Harrahy et al. 1994

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Stoneflies, *Peltoperla arcuata*	nominal concentrations of 0, 1.0, 10, 100, or 1000 ppb DFB in dechlorinated tap water for 96 hours and then transferred to glass chambers containing pesticide-free water and fed stream conditioned red maple and white oak leaves.	Survival at 10 and 1000 ppb was significantly different from controls; however, survival at 100 ppb was not significantly different from survival of controls. No behavioral changes were observed.	Harrahy et al. 1994
Mayflies, *Cyngmula subaequalis*, *Stenacron interpunctatum*, *Stenonema meririvulanum*, and *S. femaratum*	0, 0.6, 5.6, 55.7, or 557.2 ppb DFB (Dimilin 25% WP) in water for 96 hours then placed in pesticide-free water for 36-day observation period	after 4 days of exposure, mayflies were significantly lower than controls al all concentrations tested. At the lowest concentration, only about 45% survived to day 36. Many of the treated mayflies died while molting, while others died from incomplete hardening of the new cuticle. Behavioral changes observed included decreased swimming speed at higher concentrations, and no avoidance of pipet or hands during water replacement activities. Some mayflies were observed to shake sporadically before dying.	Harrahy et al. 1994
Daphnids, *Daphnia magna*	48-hour exposure to diflubenzuron (97.6% pure)	48-hour NOEC = 0.45 µg/L 48-hour EC_{50} = 7.1 µg/L (95% CI = 5.0-1.0 µg/L)	Kuijpers 1988 MRID 40840502
Fairy shrimp, *Streptocephalus sudanicus*, females	Dimilin (solvent-based, liquid ULV formulation) for 24 or 48 hours under static conditions	24-hour EC_{50} =13.3 µg/L (range = 12.8-14.0 µg/L) 48-hour EC_{50} =0.74 µg/L (range = 0.60-0.88 µg/L)	Lahr et al. 2001
Backswimmer, *Anisops sardeus*, females	Dimilin (solvent-based, liquid ULV formulation) for 24 or 48 hours under static conditions	24-hour EC_{50} =2123 µg/L (range = µg/L) 48-hour EC_{50} =1937 µg/L (range = 1800-2020 µg/L)	Lahr et al. 2001
Daphnia magna	Technical grade diflubenzuron (TH-6040)	LOEC for reproduction: 0.09 ppb	LeBlanc 1975

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Blue crabs, *Callinectes sapdidus*, embryos	acute toxicity; diflubenzuron exposure in culture plates	hatching EC_{50} =1.8 µg/L	Lee and Oshima 1998
Littoral enclosure community of mixed insects	Dimilin at nominal treatment levels of 0.7, 2.5, 7.0, or 30 µg/L to littoral enclosures	EC_{50} = 1.2 µg/L (measured concentration) NOEC = 1.0 µg/L (measured concentration) LOEC = 1.9 µg/L (measured concentration)	Liber 1995 ***In***: Moffett 1995 MRID 44386201
Littoral zooplankton community dominated by cladocera, copepoda, rotifera, and ostracoda.	Dimilin at nominal treatment levels of 0.7, 2.5, 7.0, or 30 µg/L to littoral enclosures.	Cladocera were extremely sensitive to treatment, with mean population abundances significantly reduced, compared with controls, at all four treatment levels. Mean population densities at ≥2.5 µg/L were 92 to >99% lower than mean control values by day 6 and remained at those levels through day 56. None of the decreased populations at ≥2.5 µg/L showed any sign of recovery throughout the study. Copepoda were adversely affected by treatment at all concentration levels. LOEC = 0.7 µg/L. The measured peak diflubenzuron concentration in water was 1.0 µg/L. Copepoda were significantly affected at this level, not unlike the Cladocera. The NOEC for both Claodcera and Copepoda was defined as <0.7 µg/L; however the effects at 0.7 µg/L appeared to be transistory with recovery after a single application observed within 12-29 days. Ostracoda densities were reduced at the two highest concentrations. NOEC = 2.5 µg/L Rotifera were not affected by treatment at any concentration level. NOEC = >30 µg/L.	Liber and O'Halloran 1995 ***In***: Moffett 1995 MRID 44386201 Published as Liber et al. 1996 and as O'Halloran et al. 1996
Chironomus plumosus, 4th instar larvae	Dimilin 25-WP, 48 hour exposure	EC_{50} = 0.56 mg/L	Julin and Sanders 1978

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Daphnia magna	Dimilin 25-WP® - 48 hour exposure	LC_{50} = 0.00075 mg/L (neonate) LC_{50} = 0.02345 mg/L (adult)	Majori et al. 1984
Dragonfly nymphs *Orthemis* spp., *Pantala* sp.	TH 6040 (diflubenzuron) - 168 hour exposure	LC_{50} = 50 µg/L	Miura and Takahashi 1974
Mayfly nymphs *Callibaetis* sp.	TH 6040 (diflubenzuron) - 168 hour exposure	LC_{90} = 10 µg/L	Miura and Takahashi 1974
Aedes nigromaculatum	TH 6040® (diflubenzuron) - 48 hour exposure	LC_{50} = 0.5 µg/L	Miura and Takahashi 1974
Water scavenger beetle larvae *Hydrophilus triangularis*	TH 6040® (diflubenzuron) - 48 hour exposure	LC_{50} = 100 µg/L	Miura and Takahashi 1974
Water scavenger beetle adults *Laccophilus* spp., *Thermonectus basillaris*, *Tropisternus lateralis*	TH 6040® (diflubenzuron) concentrations as high as 250 µg/L	no mortality	Miura and Takahashi 1974
Mysid shrimp,*Mysidopsis bahia*	life-cycle exposure under flow-through conditions	96-hour LC_{50} = 2.1 µg/L 21-day LC_{50} = 1.24 µg/L direct adverse effect on reproduction: the numbers of juveniles/female were significantly depressed at all nominal concentrations (0.075-0.75 µg/L)	Nimmo et al. 1979
Littoral enclosure community of mixed benthic marcroinvertebrates,predominantly, Chironomidae (midges), Oligochaeta (earthworms), and Mollusca	Dimilin at nominal treatment levels of 0.7, 2.5, 7.0, or 30 µg/L to littoral enclosures. Study duration = 71 days.	Reductions in abundance of Ephemeroptera (mayflies) and Odonata (damselflies and dragonflies) were observed at all nominal concentrations ≥2.5 µg/L. No adverse effects were observed on molluscs or earthworms at any of the four diflubenzuron test concentrations. Overall, the only benthic macroinvertebrate group that appeared to have been adversely affected by exposure to diflubenzuron was the Insecta.	O'Halloran and Liber 1995 ***In***: Moffett 1995 MRID 44386201

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Stoneflies (*Pteronarcys proteus* and *Pteronarcys arcuata*)	fed leaves from treated poplar after conditioning in stream	No effect on survival.	Perry 1995a
Blue crabs, *Callinectes sapidus*, juveniles	Dimilin WP-25 in static renewal tests	both molt stage and renewal frequency affected toxicity: LC_{50} (random molt stages) = 3.5 mg/L LC_{50} (day of molt) = 300 µg/L LC_{50} (day of molt and repeated dosing) = 18.5 µg/L	Rebach 1996
Copepods, *Eurytemora affinis*, naupli	0.78 µg/L WP25 commercial DFB (25% DFB, 75% kaolin) and filtered river water for 5 or 6 days	0% survival at >1.69 µg/L; at 0.93 µg/L survival did not differ significantly from controls.	Savitz et al. 1994
Copepods, *Eurytemora affinis*, naupli	WP25 commercial DFB (25% DFB, 75% kaolin) and filtered river water.	48-hour LC_{50} =2.2 µg/L	Savitz et al. 1994
Daphnids, *Daphnia magna*	Continuous exposure to ^{14}-C-diflubenzuron nominal concentrations of 6.3-100 ng/L (mean measured concentrations of 5.6, 14, 23, 40, or 93 ng/L) under flow-through conditions for 21 days (one generation)	50% survival at 93 ng/L[0.093 µg/L]; survival at the other test concentrations ranged from 93 to 98%, comparable to controls. significant reduction in reproduction and body length at 93 ng/L, compared with controls ($p \leq 0.05$); at other test concentrations, reproduction and growth were comparable to controls. NOEC = 40 ng/L [0.04µg/L]	Surprenant 1988 MRID 40840501
Quahog clams, *Mercenaria mercenaria*	48-hour exposure to nominal concentrations of 100 or 500 µg a.i./L (mean measured concentrations of 79, or 320 µg a.i./L) of diflubenzuron (97.6% pure)	No adverse effects on development of quahog embryos and larvae NOEC >320 µg a.i./L	Surprenant 1989 MRID 41392001

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Grass shrimp, *Palaemonetes pugio*	continuous exposure to 1-10 µg/L from inter-molt to molt (normally 7-14 days) and transfer to filtered seawater	Mortalities generally related to molt cycle with death occurring at the time of ecdysis or immediately after (LC_{50} = 0.65 µg/L); at concentrations of 7.5-10 µg/L, some shrimp did not die during the exposure period and displayed delayed progress in the molt cycle, and although these shrimp began progressing through the molt cycle when transferred to filtered seawater, they all failed to reach ecdysis and eventually died. Control shrimp were never observed in an arrested stage in the molt cycle during the experiment.	Tourat and Rao 1987 *In*: Technology Sciences Group 1998 MRID 44399307
Grass shrimp, *Palaemonetes pugio*	24-hour pulsed exposure with transfer to DFB-free medium	LC_{50} =3.4 µg/L (premolt animals D_1 - D_2)	Tourat and Rao 1987 *In*: Technology Sciences Group 1998 MRID 44399307
Grass shrimp, *Palaemonetes pugio*,	96 hours	LC_{50} =1.1 µg/L (premolt animals D_1 - D_2) very few or no mortalities among shrimp in very late premolt, early premolt, intermolt, or early postmolt stages during the 96-hour exposure.	Tourat and Rao 1987 *In*: Technology Sciences Group 1998 MRID 44399307
Horseshoe crabs, *Limulus polyphemus*, eggs	0, 5, or 50 µg/L DFB	at 5 µg/L, crabs showed a slight, but significant ($p<0.05$) delay in molt at 14 days, then molted at a rate comparable to controls and did not exhibit significant mortality. At 50 µg/L, molted at the same rate as controls but exhibited significant mortality immediately after ecdysis. Also, the prosomal width of the crabs in this group was smaller, compared with controls and crabs in the low dose group.	Weis and Ma 1987
snail *Physa* sp.	acute exposure	LC_{50} > 125 ppm	Wilcox and Coffey 1978

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Grass shrimp, *Palaemonetes pugio*, ovigerous carrying 0.5-, 1-,3-, 6-, or 8-day old embryos	continuous exposure for 4 days to 0.3-5.0 µg/L DFB in static system with transfer after exposure to DFB-free seawater for rest of the embryonic development.	No correlation between age of the embryos at exposure and either hatchability or duration of larval development; severity of abnormality did not vary with the age of the embryos except at exposure concentration of 2.5 µg/L. Larval viability was significantly ($p<0.05$) affected by the age of the embryos at the time of exposure to DFB, with older embryos more sensitive to sublethal effects of DFB.	Wilson 1997b
Grass shrimp, *Palaemonetes pugio* at different life stages (embryos, larvae, postlarvae male and female non-spawning adults, and ovigerous females.	96 hours under static renewal conditions	larvae and post-larvae most sensitive to acute toxicity of DFB with LC_{50} values of 1.44 and 1.62 µg/L, respectively; ovigerous females (hence embryos) appeared to be the most resistant to the acute toxicity of DFB with a mean LC_{50} of 6985 µg/L.	Wilson and Costlow 1987
Grass shrimp, *Palaemonetes pugio*	chronic exposure to either technical grade DFB (98.4% a.i.) Or the wettable powder (WP-25) (25% a.i.)	72-hr and 96-hr calculated LC_{50} values were similar for the two formulations of DFB (WP-25 and TG): 72-hr LC_{50} = 2.95 µg/L (TG) 72-hr LC_{50} = 2.83 µg/L (WP-25) 96-hr LC_{50} = 1.84 µg/L (TG) 96-hr LC_{50} = 1.39 µg/L (WP-25) The investigators conclude that results from studies using technical grade DFB are applicable to the WP-25 formulation without the need for a "correction factor."	Wilson and Costlow 1986

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Copepods, *Eurytemora affinis*, naupli, 24- to 48-hours old, initially	0, 0.5, 0.78, or 0.93 ppb DFB under pulse (two 6.5 exposure periods) and continuous (14-day) exposure regimens.	In pulse exposures, copepods exposed in the first 6.5 days showed a significantly lower survival rate at 0.78 and 0.93 ppb; copepods exposed during the second half of the experiment showed no significant differences in mortality, compared with controls. In the 14-day continuous exposure, survival was significantly lower at 0.78 and 0.93 ppb, but was significantly higher than that in the early pulse exposure to 0.78ppb. Effects on brood production were observed at 0.8 ppb in individuals exposed only during the copepodite stages. Significant effects on production of naupli were observed only in the first 6.5 days of pulse exposure to 0.93 ppb. At salinities of 2, 10, and 15 ppt, survival from naupilar to adult stages was significantly reduced at 0.84 ppb and none survived to adulthood at 1.7 ppb.	Wright et al. 1996

[a]Values in parentheses are 95% confidence limits.

Appendix 8: Toxicity of diflubenzuron to aquatic plants

Species	Exposure	Effects [a]	Reference
		ALGAE	
Phytoplankton communities in littoral enclosures	Dimilin at nominal treatment levels of 0.7, 2.5, 7.0, or 30 µg/L to littoral enclosures	Phytoplankton, as measured by cell size distributions and chlorophyll *a* in the enclosures, were not affected directly or indirectly by diflubenzuron treatment. No occasions of significant ($p \leq 0.05$) linear correlations between the nominal concentrations of diflubenzuron and phytoplankton measures. These results were consistent with the idea that diflubenzuron does not directly inhibit non-chitinous biota due to the specificity of its mode of action.	Moffett 1995 ***In***: Moffett 1995 MRID 44386201
Periphyton communities in littoral enclosures	Dimilin at nominal treatment levels of 0.7, 2.5, 7.0, or 30 µg/L to littoral enclosures	Late in the season (September), a 80 and 90% reduction in periphyton dry weight and 75 and 80% reduction in chlorophyll *a* at 7.0 and 30 µg/L treatment levels, respectively. Differences were statistically significant ($p=0.01$) on day 55 and nearly significant ($p=0.07$) on day 67.	Moffett 1995 ***In***: Moffett 1995 MRID 44386201
Macrophyte populations in littoral enclosures	Dimilin at nominal treatment levels of 0.7, 2.5, 7.0, or 30 µg/L to littoral enclosures	No adverse effects, direct or indirect, were observed on macrophyte species composition or total standing crop. There was no correlation between treatment concentrations and total macrophyte density throughout the study. The investigator indicates that direct effects were not anticipated because macrophytes do not have chitin.	Moffett 1995 ***In***: Moffett 1995 MRID 44386201
Blue-green algae, *Plectonema boryanum*	0.1 ppm TH-6040 in pure culture for 4 days	No growth inhibition, rapid metabolism of compound in water. Algae degraded 80T of compound in 1-hour incubation period to p-chlorophenyl urea and p-chloroaniline.	Booth and Ferrell 1977

Appendix 8: Toxicity of diflubenzuron to aquatic plants

Species	Exposure	Effects [a]	Reference
Freshwater algae *Selenastrum capricornutum*	300 µg/L diflubenzuron for 5 days	NOEC = 300 µg/L	Thompson and Swigert 1993b MRID 42940104
Freshwater algae, *Selenastrum capricornutum*	120 hour exposures; effect criteria = growth	NOEC 45 µg/L (highest concentration tested)	Hansen and Garton 1982a
Freshwater diatoms (*Navicula pelliculosa*)	380 µg/L for 5 days	NOEC = 380 µg/L	Thompson and Swigert 1993c MRID 42940105
Marine diatoms (*Skeletonema costatum*)	270 µg/L for 5 days	NOEC = 270 µg/L	Thompson and Swigert 1993d MRID 42940106
		MACROPHYTES	
Macrophyte populations in littoral enclosures	Dimilin at nominal treatment levels of 0.7, 2.5, 7.0, or 30 µg/L to littoral enclosures	No adverse effects, direct or indirect, were observed on macrophyte species composition or total standing crop. There was no correlation between treatment concentrations and total macrophyte density throughout the study.	Moffett 1995 ***In***: Moffett 1995 MRID 44386201
Duckweed (*Lemna gibba*)	190 µg/L diflubenzuron for 14 days	NOEL = 190 µg/L	Thompson and Swigert 1993a MRID 42940103

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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