



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

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TABLE OF CONTENTS

TABLE OF CONTENTS	ii
LIST OF TABLES	iv
LIST OF FIGURES	v
LIST OF APPENDICES	v
ACRONYMS, ABBREVIATIONS, AND SYMBOLS	vi
COMMON UNIT CONVERSIONS AND ABBREVIATIONS	viii
CONVERSION OF SCIENTIFIC NOTATION	ix
EXECUTIVE SUMMARY	x
1. INTRODUCTION	1-1
2. PROGRAM DESCRIPTION	2-1
2.1. Overview	2-1
2.2. Chemical Description and Commercial Formulations	2-1
2.3. Use Statistics	2-3
3. HUMAN HEALTH RISK ASSESSMENT	3-1
3.1. HAZARD IDENTIFICATION	3-1
3.1.1. Overview.	3-1
3.1.2. Epidemiology Studies.	3-1
3.1.3. Mechanism of Action.	3-7
3.1.4. Acute Oral Toxicity.	3-8
3.1.5. Subchronic or Chronic Systemic Toxic Effects.	3-9
3.1.6. Effects on Nervous System.	3-9
3.1.7. Effects on Immune System.	3-9
3.1.8. Effects on Endocrine System.	3-11
3.1.9. Reproductive and Teratogenic Effects.	3-11
3.1.10. Carcinogenicity and Mutagenicity.	3-11
3.1.11. Irritation and Sensitization (Effects on the Skin and Eyes).	3-11
3.1.12. Systemic Toxic Effects from Parenteral Exposure.	3-12
3.1.13. Inhalation Exposure.	3-13
3.1.14. Impurities.	3-13
3.1.15. Inerts.	3-14
3.2. EXPOSURE ASSESSMENT	3-16
3.2.1. Overview.	3-16
3.2.2. General Issues.	3-16
3.2.3. Workers.	3-17
3.2.4. General Public.	3-18
3.3. DOSE-RESPONSE ASSESSMENT	3-19
3.3.1. Overview.	3-19
3.3.2. Existing Guidelines.	3-19
3.3.3. Human Data.	3-20
3.3.4. Animal Data.	3-23
3.3.5. Values Used in Risk Characterization.	3-25
3.4. RISK CHARACTERIZATION	3-27
3.4.1. Overview.	3-27
3.4.2. Irritant Effects.	3-28

TABLE OF CONTENTS *(continued)*

3.4.3. Serious Adverse Effects.	3-29
3.4.4. Groups at Special Risk.	3-31
3.4.5. Cumulative Effects and Connected Actions.	3-32
4. ECOLOGICAL RISK ASSESSMENT	4-1
4.1. HAZARD IDENTIFICATION	4-1
4.1.1. Overview.	4-1
4.1.2. Toxicity to Terrestrial Organisms.	4-2
4.1.2.1. Mammals	4-2
4.1.2.2. Birds	4-3
4.1.2.3. Terrestrial Invertebrates	4-3
4.1.2.4. Terrestrial Plants (Macrophytes)	4-7
4.1.2.5. Terrestrial Microorganisms	4-8
4.1.3. Aquatic Organisms.	4-8
4.1.3.1. Fish	4-8
4.1.3.2. Amphibians	4-8
4.1.3.3. Aquatic Invertebrates	4-8
4.1.3.4. Aquatic Plants	4-9
4.2. EXPOSURE ASSESSMENT	4-10
4.2.1. Overview.	4-10
4.2.2. Terrestrial Animals.	4-10
4.2.2.1. Terrestrial Vertebrates	4-10
4.2.2.2. Terrestrial Invertebrates	4-11
4.2.2.3. Other Terrestrial Species	4-16
4.2.3. Aquatic Organisms.	4-16
4.3. DOSE-RESPONSE ASSESSMENT	4-18
4.3.1. Overview.	4-18
4.3.2. Toxicity to Terrestrial Organisms.	4-18
4.3.2.1. Terrestrial Vertebrates	4-18
4.3.2.2. Terrestrial Invertebrates	4-19
4.3.3. Aquatic Organisms.	4-19
4.3.3.1. Fish	4-21
4.3.3.2. Invertebrates	4-22
4.4. RISK CHARACTERIZATION	4-23
4.4.1. Overview.	4-23
4.4.2. Terrestrial Organisms.	4-23
4.4.2.1. Terrestrial Vertebrates	4-23
4.4.2.2. Terrestrial Invertebrates	4-24
4.4.3. Aquatic Organisms.	4-25
5. REFERENCES	5-1

TABLE OF CONTENTS *(continued)*

LIST OF TABLES

Table 2-1: Commercial formulations of <i>B.t.k.</i> that may be used in USDA Gypsy Moth Programs	Tables-1
Table 2-2: Use of <i>B.t.k.</i> from 1995 to 2001 for Suppression, Eradication, and Slow the Spread	Tables-2
Table 3-1: Epidemiology Studies on <i>B.t.k.</i> Formulations	Tables-3
Table 3-2: Publically available information on inerts used in <i>B.t.k.</i> formulations	Tables-4
Table 3-3: Overview of exposure data for workers and members of the general public	Tables-5
Table 3-4: Post-spray symptoms reported by ground-spray workers and controls	Tables-6
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	Tables-7
Table 3-6: Self-reported symptoms in individuals before and after the aerial application of <i>B.t.k.</i>	Tables-8
Table 3-7: Exposure conversions for mice and humans with effects noted in mice after intranasal instillations	Tables-9
Table 3-8: Risk characterization for serious health effects from exposure to <i>B.t.k.</i> ..	Tables-10
Table 4-1: Mortality in species subject to foliage treated with Foray 48B at 89 BIU/ha	Tables-11
Table 4-2: Mortality in species subject to foliage treated with Dipel 8AF at 99 BIU/ha	Tables-12
Table 4-3: Summary of exposures used in ecological risk assessment	Tables-13
Table 4-4: Summary of toxicity values used in ecological risk assessment	Tables-14
Table 4-5: Data used in dose-response assessment for non-target insects	Tables-15
Table 4-6: Risk characterization for ecological risk assessment of <i>B.t.k.</i>	Tables-16

TABLE OF CONTENTS (*continued*)

LIST OF FIGURES

- 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 Figures-1
- 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 LD₅₀ . . . Figures-2
- Figure 4-1: Dose-response assessment for non-target terrestrial invertebrates Figures-3
- Figure 4-2: Distribution of sensitivity in various non-target lepidoptera Figures-4

LIST OF 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

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
<i>B.t.k.</i>	<i>Bacillus thuringiensis</i> var. <i>kurstaki</i>
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 _{oc}	organic carbon partition coefficient
K _{ow}	octanol-water partition coefficient
K _p	skin permeability coefficient
L	liter
LdNPV	gypsy moth (<i>Lymantria dispar</i>) 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
≥	greater than or equal to
<	less than
≤	less than or equal to
=	equal to
≈	approximately equal to

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

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

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

CONVERSION OF SCIENTIFIC NOTATION

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

EXECUTIVE SUMMARY

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/m³ × 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 LD₅₀ 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 mortality 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 persist 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 been 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/m³ 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 10⁷

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, Cry1Aa, Cry2Aa, and Cry2Ab 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 to 10 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 Javelin (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 Bernstien 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 1 workers (~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 1 is 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. reagenic (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/m³ 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 Auckland 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 m² 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 m². Petrie et al. (2003) do specify that their survey was conducted after three aerial sprays. While it is possible 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; Drobniowski 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 Shaddock 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 (Drobniowski 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 an 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 LD₅₀ —i.e., 4 of 20 mice at 10² spores/mouse, 8 of 20 mice at 10⁴ spores/mouse, and 14 of 20 mice at 10⁷ 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 LD₅₀ with no *B.t.* exposure. In addition, weight loss was observed in mice treated with influenza virus at 2% of the LD₅₀ and this correlated well with the dose of *B.t.* 3a3b used to infect the mice suggesting that a low inoculum 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 10⁴ spores/mouse and 10⁷ spores/mouse but not at 10² 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 inoculum 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 development 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 accidentally 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 accidentally 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 LD₅₀ 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 10⁸ [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 10², 10⁴, and 10⁶ cfu/mouse caused only local inflammation. A dose of 10⁸ 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 misled 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/m³) 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/m³ 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/m³. 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/m³, 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/m³— 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/m³ × 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 LD₅₀ 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 ($p \approx 0.0077$) and throat irritation ($p \approx 0.0079$) 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 $\text{cfu}/\text{m}^3 \times \text{hours}$ 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 patterns 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/m³ —i.e., about 16 million cfu/m³. 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:

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

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/m³ 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/m³ 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 $[(0.32 - 0.16) \div (1 - 0.16) = 0.1904]$ 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 to *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 LD₅₀. In mice not exposed to the influenza virus, the only effect noted was local inflammation. 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 LD₅₀ 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 LD₅₀ 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 log₁₀ 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/ $\text{m}^3 \times \text{hours}$ [3.13×10^{12} cfu/ $\text{m}^3 \times 4$ 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 inflammation. 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/ $\text{m}^3 \times \text{hours}$ 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., $70 \text{ kg} \div 0.02 \text{ kg}$. 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/ $\text{m}^3 \times \text{hour}$

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/m³ × hours are likely to result in local inflammation 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/m³. The apparent lack of a strong dose-response relationship in humans is consistent with the wide dose range leading to local inflammation 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/m³ (1.58×10^7 cfu/m³) 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/m³ × hours. In other words, a worker would need to be exposed to 1.58×10^7 cfu/m³ for about 37 days to reach a cumulative dose of 1.4×10^{10} cfu/m³ × hours [$(1.4 \times 10^{10}$ cfu/m³ × hours) ÷ 1.58×10^7 cfu/m³ = 886 hours or about 37 days]. The highest cumulative exposure reported by Cook (1994) is $>3 \times 10^8$ cfu/m³ × hours, a factor of about 50 below the highest estimated non-lethal exposure of 1.4×10^{10} cfu/m³ × hours based 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/m³ × 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/m³ × 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/m³ × 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 LD₅₀ 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, exposure levels 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 than 1000 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 [$1000 \div 100$

cfu/m³] and workers exposed to 15.8×10^6 cfu/m³ (i.e., workers in the study by Cook 1994) would have a hazard quotient of 158,000 [$15,800,000 \div 100$ cfu/m³], 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 inflammation, 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/m³ × hour could be adopted directly as a NOAEL with a 10-fold higher dose [1×10^{11} cfu/m³ × 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 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/m³ × 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/m³ × 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/m³. 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/m³ 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/m³ × 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/m³. The hazard quotient is given in the last column as the cumulative exposure divided by the estimated human NOAEL of 1×10^{10} cfu/m³ × 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/m³. The use of the LD₁₀ 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/m³. 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/m³ × hours. More plausible estimates, based on only a 2-hour rather than a 24-hour duration, range from 1200 to 30,000 hours × cfu/m³ 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/m³ × 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 be 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 \times cfu/m³—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 be 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 in Appendix 1, *B.t.k.* may persist—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 1 year 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 (amalachier, 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 (1st to 5th instar), a non-target beneficial species, and the cabbage looper (*Trichoplusia ni*), a target species (1st 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 (LC₅₀ values of 427 to 575 BIU/ha) and later instars being extremely sensitive (LC₅₀ values of 19 and 26 BIU/ha). The sensitive instars are about as sensitive to the *B.t.k.* formulations as the target species (LC₅₀ 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 ferveridaria* [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/cm³ 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 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/m³, 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/m³ 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 m³/hour. Taking the concentrations of 100 to 5000 cfu/m³ 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/m³ × 0.0000014 m³/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 [$0.480 \text{ mg/g vegetation} \times 3.5 \text{ g} \div 0.02 \text{ kg}$].

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/m². Under the assumption that the *B.t.k.* formulation is sprayed over a shallow (1 cm deep) puddle with a surface area of 1 square meter or 10,000 cm², 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 [$224.2 \text{ mg} \div 10,000 \text{ mL}$]. Thus, the *B.t.k.* dose to the 20 g mammal is approximately 3.3 mg/kg [$0.022 \text{ mg/mL} \times 3 \text{ mL} \div 0.02 \text{ kg}$].

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 m². Thus, at an application rate of 2 lbs/acre or 223.4 mg/m², the maximum dose that could be deposited on a 20 g mammal is about 97 mg/kg body weight [$224.2 \text{ mg/m}^2 \times 0.0086509 \text{ m}^2 \div 0.02 \text{ kg}$]. 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 [$84 \text{ mg/kg} + 3.3 \text{ mg/kg} + 97 \text{ mg/kg} = 184.3 \text{ mg/kg bw}$].

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 use 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 Eilersieck, 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/m². At a depth of 1 m, 244.2 mg of formulation would be deposited into 1 m³ 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 [$4.7 \text{ L/ha} \times 0.2642 \text{ gallon/L} \times 16 \text{ BIU/gallon} \times 0.4047 \text{ acres/ha} = 8.0405 \text{ BIU/acre}$], 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 [$63,000 \text{ cfu/L} \div 8 \text{ BIU/acre}$]. 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 [$7875 \text{ cfu/L per BIU/acre} \times 40 \text{ BIU/acre} = 315,000 \text{ cfu/L}$].

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 LD₅₀ value of about 21 BIU/ha. Tolerant species, comprised of some lepidoptera and other kinds of terrestrial insects, have an LD₅₀ value of about 590 BIU/ha, which is approximately 28 times greater than the LD₅₀ 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/m³ 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 with 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 cabbage 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 LD₅₀ value of about 21 BIU/ha and consist entirely of lepidoptera. The tolerant species have an LD₅₀ of about 590 BIU/ha, which is approximately 28 times greater than the LD₅₀ 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:

$$P = (P^* - C) / (1 - C)$$

where **P** is the proportion responding that is attributable to the agent, **P*** is the observed proportion responding in the group exposed to the agent, and **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:

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

where 0 and 1 are the mean and standard deviation of the standard normal curve, and **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:

$$Y = a + bx$$

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

$$Y = a + bx + cS$$

where c is the coefficient for the sensitivity variable, S , and the other terms are as defined above.

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

$$Y = -1.48 + 2.34 x + 3.36 S$$

with a highly significant 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 Y equal to 5 and S equal to 1. With these substitutions, the value of 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 Y equal to 5 and S equal to 0 to yield a \log BIU/ha of about 2.77, corresponding to an LD_{50} of about 590 BIU/ha [$10^{2.77}$]. Thus, the relative potency of *B.t.k.* to sensitive species is about 28, relative to tolerant species [$590 \text{ BIU/ha} \div 21 \text{ BIU/ha}$].

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 fuscum/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 (Trichoptera). 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/m³, 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/m³) and the breathing rate of the mouse (0.0000336 m³/day). The resulting hazard index of 2×10^{-8} — $0.168 \text{ cfu/mouse} \div 10^7 \text{ cfu/mouse}$ 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:

$$Y = -1.48 + 2.34 x + 3.36 S$$

where Y is the probit response, x is the common log of the application rate in BIU/ha, and 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 be among the most tolerant lepidoptera. Based on the study by Peacock et al. (1998), owl 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/m² 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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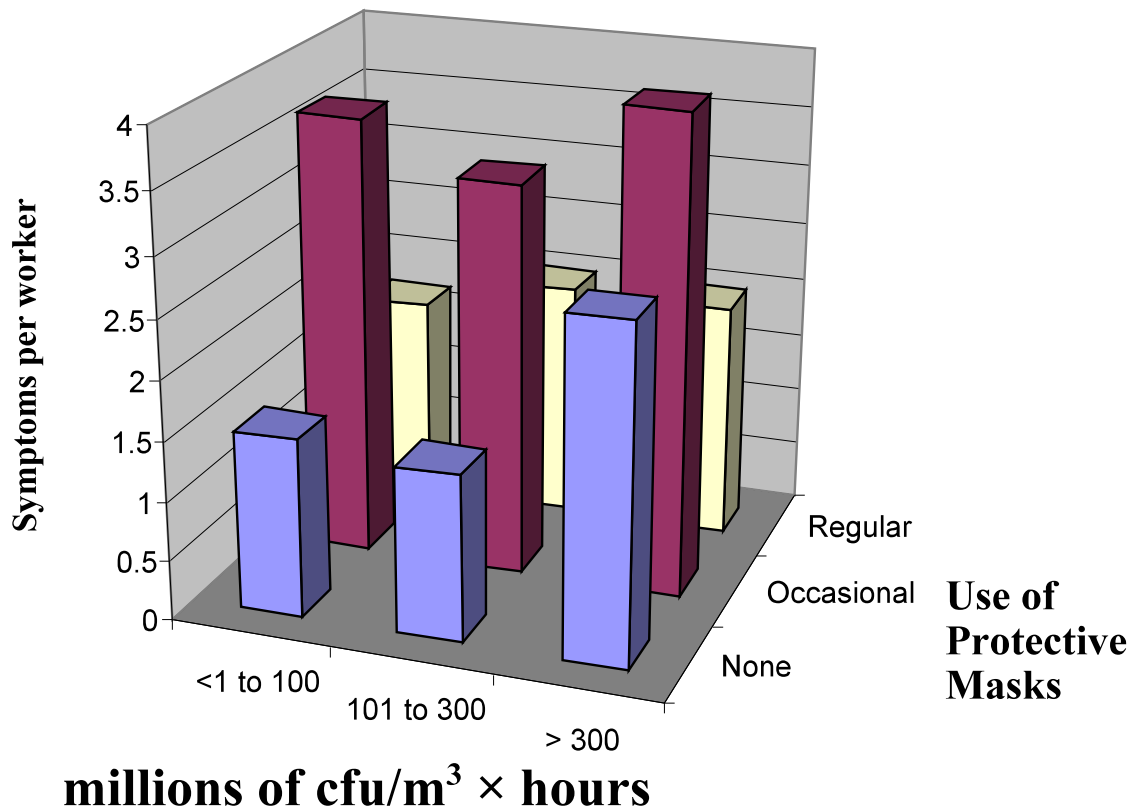


Figure 3-1: Number of symptoms per worker based on total exposure to *B.t.k.* (millions of cfu hours) and the use of protective masks (data from Cook 1994 as summarized in Table 3-6 of this risk assessment)

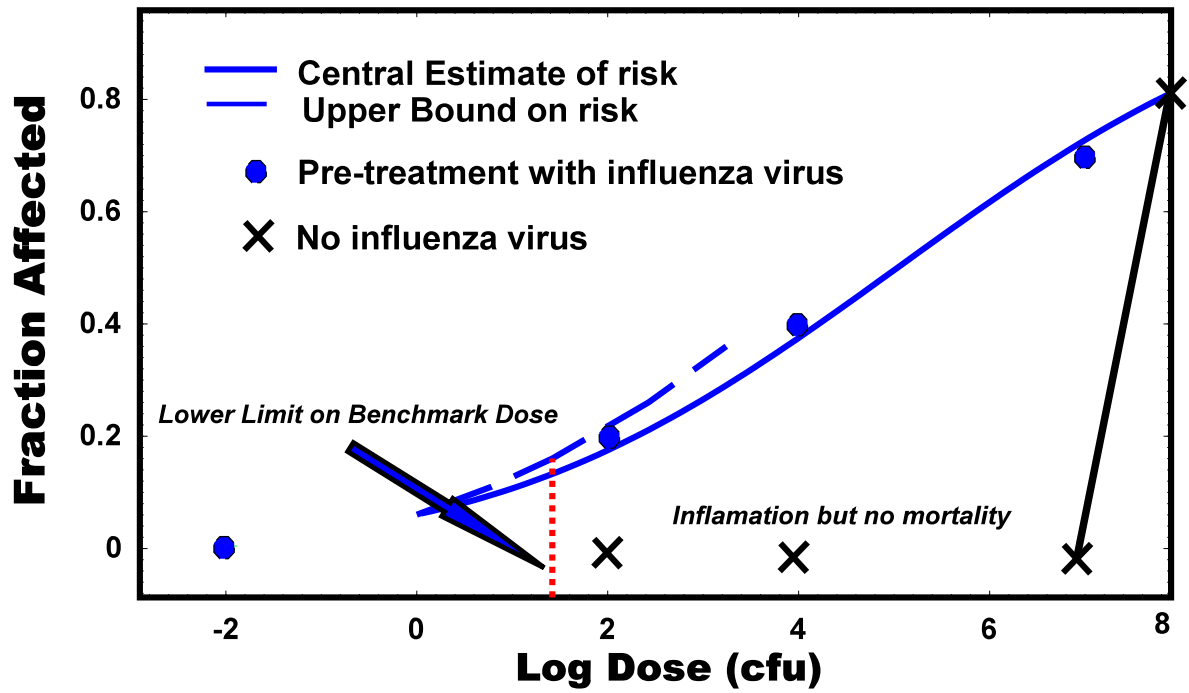


Figure 3-2: Dose-response relationships in mice after intranasal administration of *B.t.k.* with or without previous challenge with influenza virus at 4% of the LD₅₀ (data from Hernandez et al. 1999 and 2000).

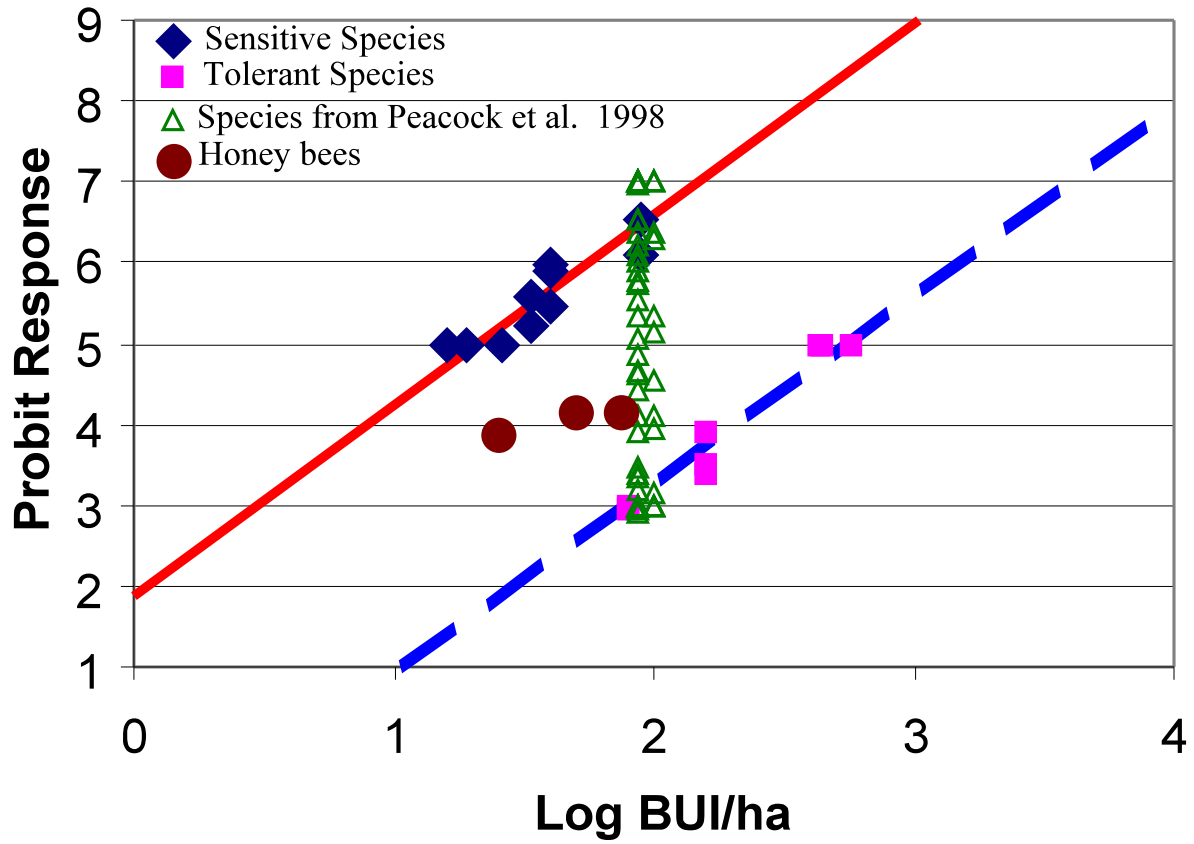


Figure 4-1: Dose-Response Assessment for non-target terrestrial invertebrates.

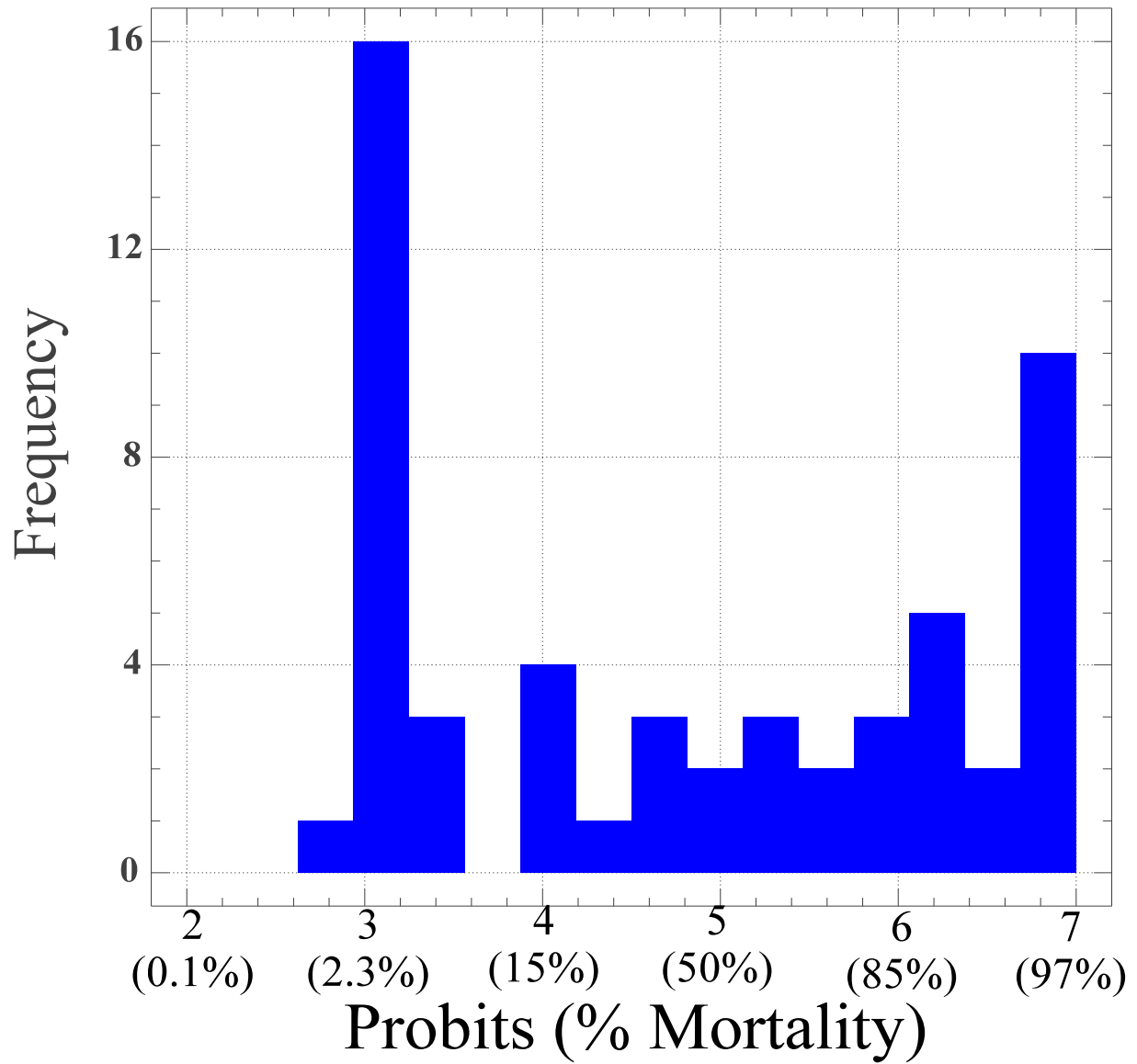


Figure 4-2: Distribution of sensitivity in various non-target lepidoptera (data from Peacock et al. 1998)

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 <i>B.t.k.</i> in clinical samples. Seven <i>B.t.k.</i> 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. <i>B.t.k.</i> not implicated as disease agent. Cellular fatty acid profiles of <i>B.t.k.</i> cultures from humans as well as plants differed from <i>B.t.k.</i> in formulation. Some workers involved in ground applications evidenced nasal swabs positive for <i>B.t.k.</i> for up to 120 days after application. Respiratory and dermal irritation in workers.	Cook (1994); Noble et al. (1992)
Javelin (<i>B.t.k.</i> 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 <i>B.t.k.</i> 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. Increase in incidence of <i>B.t.k.</i> HD-1 from nasal swabs after <i>B.t.k.</i> spray. Relatively few <i>B.t.k.</i> HD-1 identified in water (2.9%).	Pearce et al. 2002 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 <i>B.t.k.</i> 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 <i>B.t.k.</i> inside spray area during spray.	Valadares de Amorim et al. 2001
484-551 cfu/m ³	95% of samples positive for <i>B.t.k.</i> outside spray area during spray.	Valadares de Amorim et al. 2001

¹See Table 3-1 for a description of the epidemiology studies.

²Excluding 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 (%)		<i>p</i> -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/m³ × 10⁶ × 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

¹The 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 $0.05 \div 25 = 0.002$]

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	inflammation, 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 $\frac{B_{\text{wk}}}{1.0496}$. Note that 0.0014 L/hour is equivalent to 0.0000014 m³/hour [1 m³ = 1000 L] or 0.0000336 m³/day.

⁽²⁾ cfu/mouse × 70 kg/0.02 kg.

⁽³⁾ Based on a human breathing rate for moderate activity of 2.5 m³/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		<i>p</i> -value ³
			No. Alive	No. Dead	No. Alive	No. Dead	
Papilionidae, Swallowtail Butterflies	<i>Papilio glaucus</i>	1-3	10	0	0	20	<0.00001
Nymphalidae, Danaid and Brown Butterflies	<i>Speyeria diana</i>	2-3	10	0	1	15	<0.00001
	<i>Limenitis arthemis astyanax</i>	n/n-1	10	0	0	20	<0.00001
	<i>Astercampa clyton</i>	4-5	21	1	1	40	<0.00001
Geometridae, Looper Butterflies	<i>Alsophila pometaria</i>	n	19	1	11	7	0.0164
	<i>Phiglia titea</i>	n/n-1	20	0	43	7	0.1801
	<i>Euchlaena obtusaria</i>	n-1	12	0	18	0	1
	<i>Ennomos magnaria</i>	1	22	1	0	66	<0.00001
	<i>Ennomos magnaria</i>	1	17	14	0	27	<0.00001
	<i>Lambdina fervidaria</i>	1	17	1	10	26	<0.00001
	<i>Eutrapela clemataria</i>	H ²	20	0	4	31	<0.00001
	<i>Prochoerodes transversata</i>	2	19	1	28	13	0.0237
Lasiocampidae, Lappet Moths	<i>Malacosoma disstria</i>	2	23	4	4	26	<0.00001
	<i>Malacosoma disstria</i>	n	20	0	1	44	<0.00001
Saturniidae, Silk Moths	<i>Hemileuca maia</i>	H	47	0	5	53	<0.00001
	<i>Hemileuca maia</i>	1	70	1	48	312	<0.001
	<i>Hemileuca maia</i>	1	20	0	0	51	<0.00001
	<i>Hemileuca maia</i>	2	109	1	0	111	<0.00001
	<i>Antheraea polyphemus</i>	1	16	4	3	57	<0.00001
	<i>Actias luna</i>	1	26	14	0	96	<0.00001
Lymantriidae, Tussuck Moths	<i>Dasychira obliquata</i>	4	20	0	27	1	0.9999
Noctuidae, Owlet moths	<i>Amphipyra pyramidoides</i>	n-1	19	2	6	24	<0.00001
	<i>Amphipyra pyramidoides</i>	n-1	20	0	11	37	0.0001
	<i>Xystocheilus rufago</i>	1,2	28	0	12	21	<0.00001
	<i>Psaphida rolandi</i>	n-1	19	1	18	22	0.0001
	<i>Psaphida resumens</i>	1,2	20	0	9	41	<0.00001
	<i>Egira alternans</i>	1	20	5	22	27	0.0059
	<i>Egira alternans</i>	2-3	18	0	35	2	1
	<i>Zale aeruginosa</i>	H	12	0	19	11	0.0173
	<i>Eupsilia vinulenta</i>	n-1/n-2	20	0	19	1	0.9999
	<i>Eupsilia vinulenta</i>	n-1/n-2	20	0	43	1	0.9999
	<i>Sericaglaea signata</i>	4	18	0	48	0	1
	<i>Metaxaglaea semitaria</i>	n	20	0	51	1	0.9999
	Noctuidae, Owlet moths (continued)	<i>Chaetaglaea sericea</i>	n-1	20	0	20	0
<i>Chaetaglaea sericea</i>		n-1	19	0	48	1	0.9999
<i>Sunira biclorago</i>		n/n-1	20	0	45	3	0.5498
	<i>Sunira biclorago</i>	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		<i>p</i> -value ³
			No. Alive	No. Dead	No. Alive	No. Dead	
	<i>Xylotype capax</i>	n-1	19	1	48	0	0.2941
	<i>Orthosia alurina</i>	n-2	19	1	29	0	0.9999
	<i>Orthosia alurina</i>	n-1	18	0	30	7	0.0823
	<i>Orthosia hibisci</i>	n-1	20	0	39	0	1
	<i>Abagrotis alternata</i>	n/n-1	29	0	50	0	1
	<i>Abagrotis alternata</i>	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		<i>p</i> -value ³	Comparison to Foray
			No. Alive	No. Dead	No. Alive	No. Dead		
Geometridae, Looper Butterflies	<i>Asterocampa clyton</i>	4,5	21	1	2	20	<0.00001	
	<i>Alsophila pometaria</i>	n	19	1	11	21	<0.00001	Match
	<i>Ennomos magnaria</i>	1	17	14	0	47	<0.00001	Match
Lasiocampidae, Lappet Moths	<i>Malacosoma disstria</i>	2	23	4	0	28	<0.00001	Match
Lymantriidae, Tussuck Moths	<i>Dasychira obliquata</i>	4	20	0	26	0	1	Match
Noctuidae, Owlet moths	<i>Catocala vidua</i>	1	17	2	0	31	<0.00001	
	<i>Amphipyra pyramidoides</i>	n-1	19	2	3	35	<0.00001	Match
	<i>Lithophane grotei</i>	n-1/n-2	20	0	22	28	<0.00001	
	<i>Lithophane unimoda</i>	n-1	19	1	38	9	0.1423	
	<i>Eupsilia vinulenta</i>	n-2	20	0	19	9	0.0063	No match, different instars
	<i>Chaetagnathia sericea</i>	n-1	20	0	30	0	1	Match
	<i>Sunira biclorago</i>	n/n-1	20	0	41	0	1	Match
	<i>Orthosia alurina</i>	n-2	19	1	14	4	0.1698	Match
<i>Abagrotis alternata</i>	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 [\approx 8.4 BIU/acre] LD ₅₀ Tolerant Species: 590 BIU/ha [\approx 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 \times 10 ⁷ cfu/L – LOEC Tolerant Species: 1000 mg formulation/L or 2.5 \times 10 ¹⁰ cfu/L – NOEC	4.3.3.1.
Aquatic Invertebrates	Sensitive Species: 0.45 mg/L or 6.24 \times 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 <i>B.t.k.</i>	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, 5th 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 <i>B.t.k.</i> 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: $Y = -1.48 + 2.34 x + 3.36 S$. In this equation, Y is the probit response, x is the common log of the application rate in BIU/ha, and 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 <i>B.t.k.</i> 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, <i>B.t.k.</i> 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
<i>B.t.k.</i> (NOS) from Novo Nordisk	Rats, SPF Wistar, 4M/4F, 1 mL dose (cfu counts in dose illegible on fiche). Gavage	No mortality or signs of toxicity. No <i>B.t.k.</i> found in blood. <i>B.t.k.</i> in feces and organs dropped by a factor of 100 in 24 hours.	Harde 1990a
<i>sB.t.k.</i> 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. <i>B.t.k.</i> not found in blood of any animal. <i>B.t.k.</i> 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
<i>B.t.k.</i>	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: <i>B.t.k.</i> 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 at 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.			
<i>B.t.k.</i> formulation	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
<i>B.t.k.</i> formulation	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 (1×10 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 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
<i>B.t.k.</i> 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, <i>B.t.k.</i> could be detected in cultures from the treated eye.	Meher et al. 2002
INHALATION			
<i>B.t.k.</i> (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. (3.13×10 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. <i>B.t.k.</i> 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 (3×10^9 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 (4×10^8 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 <i>B.t.k.</i> aggravated a preexisting disease. Over 167 days, a complete elimination of the test organism from all tissues except the spleen, which on average contained 3×10^2 <i>B.t.k./g</i> at the end of the study.	Berg 1990
<i>B.t.</i> 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
<i>B.t.</i> 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
<i>B.t.</i> 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
<i>B.t.</i> 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, <i>B.t.k.</i> (Chen and Macuga 1990o,p,q)			

Appendix 1: Toxicity in Mammals			
Product	Species/Exposure	Observations	Reference
<i>B.t.k.</i> 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 <i>B.t.k.</i> in Christensen (1991c).			
FIELD STUDIES			
<i>B.t.k.</i> (Dipel 8L and red dye)	Masked shrew (<i>Sorex cinereus</i>) 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 <i>S. cinereus</i> ; 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 from 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
<i>B.t.k.</i> (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 <i>B.t.</i> strain was not identified (Robbins 1991a,b). Omitted studies of Abbott ABT-6305 in this and other tables. Abbott ABT-6305 is <i>B.t. aizawai</i> (www.epa.gov/pesticides/foia/reviews/006403.htm).			
Appendix 2: Toxicity in Birds			
Product	Species/Exposure	Observations	Reference
ORAL			
<i>B.t.</i> EG2348	Bobwhite Quail, 3333mg/kg gavage	No mortality or signs of toxicity/pathogenicity.	Beavers et al. 1988a
<i>B.t.</i> 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 <i>B.t.k.</i>	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 <i>B.t.k.</i>	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 <i>B.t.k.</i>	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 <i>B.t.k.</i>	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
<i>B.t.</i> 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
<i>B.t.</i> 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 <i>B.t.k.</i> Omitted Beavers 1991a,b on <i>B.t.</i> Abbott ABG-6305. This is <i>B.t.a.</i>			
FIELD STUDIES			
<i>B.t.k.</i> Thuricide 23LV with Rhoplex sticker	Black-throated blue warblers (<i>Dendroica caerulesceus</i>), 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 <i>neotropical migrant bird species are probably limited periodically by food when breeding in north-temperate habitats.</i>	Rodenhouse and Holmes 1992
<i>B.t.k.</i> (NOS)	Hooded warbler (<i>Wilsonia citrina</i>) on two treatment plots in the Arkansas Ozards following two applications of <i>B.t.</i> in 1994	<i>B.t.k.</i> application appeared to have only minimal adverse effects on reproduction, in as much as the decreased numbers of lepidopteran 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
<i>B.t.k.</i> (NOS)	Chestnut-backed and black-capped chickadees (<i>Parus rufescens</i> , and <i>P. atricapillus</i>), application of unspecified product at 60 BIU/ha in Portland, OR area and surrounding counties.	No effects on growth rate of fledgling success in 1 st year. Reduced fledgling success 2 nd 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
<i>B.t.k.</i> , 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 (<i>Dendragapus canadensis</i>). 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
<i>B.t.k.</i> , 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 (<i>Pipilo maculatus</i>), 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
<i>B.t.k.</i> (Thuricide 16B; Dipel WP, with or without chitinase)	Spruce budworm (<i>Choristoneura fumiferana</i>) 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
<i>B.t.k.</i> (NOS)	32 Species of Lepidoptera on tobacco brush (<i>Ceanothus velutinus</i>) treated with 20 BIU/ha (product not specified) in program to control spruce budworm (<i>Choristoneura occidentalis</i>) 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
<i>B.t.k.</i> (NOS)	35 Species belonging to 10 families in the guild of nontarget leaf-feeding Lepidoptera (caterpillars) on Garry oak (<i>Quercus garryana</i>) 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 <i>B.t.k.</i> 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
<i>B.t.k.</i> 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
<i>B.t.k.</i> (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 (<i>Orthosia hibisci</i> , <i>Protorthodes rufula</i> , <i>Perizoma curvilinea</i>) eliminated from site? Overall, moth diversity unaffected.	Crawford et al. 1993

Appendix 3: Toxicity in Non-target Lepidoptera			
Product	Species/Exposure	Observations	Reference
<i>B.t.k.</i> (NOS)	Micro-and Macro-Lepidoptera exposed to 89 BIU/ha (36 BIU/acre) in 50 acre plots of oak woodland in Rockbridge County, VA	<p>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 <i>B.t.k.</i> 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.</p> <p>6/8 most common macro-Lepidoptera species trapped under burlap bands were reduced by treatment. Three of these species were nearly absent in treated plots (<i>Satyrium calanus</i>, <i>Malacosoma disstria</i>, <i>Orthosia rubescens</i>). Other less common species appeared to be significantly less on treated plots. <i>Dasychira obliquata</i> was not affected apparently. Noctuidae also lower in 1993.</p>	Peacock et al. 1994
<i>B.t.k.</i> (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	<p>Four treatments: control; <i>B.t.</i> sprayed without gypsy moth; <i>B.t.</i> with gypsy moth; gypsy moth alone (defoliated).</p> <p>Total larval abundance reduced following <i>B.t.k.</i> application in 1991. No effects of <i>B.t.k.</i> and gypsy moth on several Lepidoptera.</p> <p>Short-term effects of <i>B.t.k.</i> on non-target lepidoptera are detrimental but longer term effects are beneficial.</p> <p>Minor effect on some species of lepidoptera consumed by bats (Noctuidae and Notodontidae).</p>	Sample et al. 1996

Appendix 3: Toxicity in Non-target Lepidoptera			
Product	Species/Exposure	Observations	Reference
<i>B.t.k.</i> (Foray 48B)	<p>Karner blue butterfly (<i>Lycaeides melissa samuelis</i>) larvae (early and late instars) reared on wild lupine foliage treated in laboratory bioassay with <i>B.t.k.</i> at rate of 30-37 or 90 BIU/ha for 7 days.</p> <p>A concurrent laboratory bioassay involving gypsy moth 2nd instars on similarly treated white oak for 7 days.</p>	<p>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.</p> <p>Survival rates for gypsy moth larvae were: 80% for controls; 33% for low-dose treatment, and 5% for high-dose treatment.</p> <p>Investigators conclude that the Karner blue is both phenologically and physiologically susceptible to <i>B.t.</i> used for gypsy moth suppression, although the larval generation at risk and extent of phenological overlap may vary from year to year.</p>	Herms et al. 1997
<i>B.t.k.</i> (Dipel: wettable powder)	Mulberry silkworm (<i>Bombyx mori</i>) larvae exposed to laboratory concentrations of 1×10 , 1×10^2 , 1×10^3 , 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , or 1×10^9 spore/mL applied to mulberry leaves	<p>LC₅₀ = 1.40×10 spores/L (larval instar I) LC₅₀ = 4.20×10^2 spores/L (larval instar II) LC₅₀ = 1.0×10^3 spores/L (larval instar III) LC₅₀ = 2.0×10^5 spores/L (larval instar IV) LC₅₀ = 6.3×10^6 spores/L (larval instar II)</p> <p>Larval mortality was dose-dependent with highest % mortality observed at highest concentrations of <i>B.t.</i> 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 <i>B.t.</i> were associated with decreased pupation, greater pupal mortality, increased incidences of malformed adult emergence and lower emergence of normal adults in all instars.</p>	Jayanthi and Padmavathamma 1997

Appendix 3: Toxicity in Non-target Lepidoptera			
Product	Species/Exposure	Observations	Reference
<i>B.t.k.</i> (Foray 48B)	Swallowtail butterflies (<i>Papilio glaucus</i> and <i>Papilio canadensis</i>) and promethea moth (<i>Callosamia promethea</i>) (1 st and 2 nd instars of the three nontarget species) exposed to Foray 48B applied at a rate of 40 BIU/ha to individual trees using a <i>B.t.-dedicated</i> 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
<i>B.t.k.</i> (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 <i>P. glaucus</i> 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: <i>Lambdina fervidaria</i> [geometrid], <i>Heterocampa guttivitta</i> [notodontid], and <i>Achatia distincta</i> [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: <i>Incisalia fotis</i> (Desert Elfin butterfly) and <i>Callophrys sheridanii</i> (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
<i>B.t.k.</i> (Dipel-HG) potency of 4320 IU/mg	Cinnabar moth (<i>Tyria jacobaeae</i>) larvae (1 st - 5 th 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 (<i>Trichoplusia ni</i>) used as positive control.	LC ₅₀ = 26 BIU/ha (4 th instar) (95% CI = 9.6-62 BIU/ha) LC ₅₀ = 19 BIU/ha (5 th instar) (95% CI = 5.9-44 BIU/ha) LC ₅₀ = 16 BIU/ha (<i>Trichoplusia ni</i>) (95% CI = 5.6-30 BIU/ha) Treatment had little effect on 1 st through 3 rd instar survival) – LC ₅₀ values of 427 to 575 BIU/ha. See text for discussion.	James et al. 1993
<i>B.t.k.</i> (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
<i>B.t.k.</i> HD-1 strain (Thuricide 32 LV)	White-marked tussock moth (<i>Orgyia leucostigma</i>) larvae (early 3 rd instar) via dietary exposure	LC ₅₀ = 12 IU/mL diet (95% CI = 9-13 IU/mL)	Frankenhuyszen et al. 1992
<i>B.t.k.</i> HD-1 strain (Thuricide 32 LV)	Eastern hemlock looper (<i>Lambdina fiscellaria fiscellaria</i>) larvae (early 3 rd instar) via dietary exposure	LC ₅₀ = 162 IU/mL diet (95% CI = 129-343 IU/mL)	Frankenhuyszen et al. 1992
<i>B.t.k.</i> HD-1 strain (Thuricide 32 LV)	Jack pine budworm (<i>Choristoneura pinus</i>) larvae via dietary exposure	LC ₅₀ = 145 IU/mL diet (95% CI = 121-169 IU/mL)	Frankenhuyszen et al. 1992
<i>B.t.k.</i> HD-1 strain (Thuricide 32 LV)	Western spruce budworm (<i>Choristoneura occidentalis</i>) larvae via dietary exposure	LC ₅₀ = 11 IU/mL diet (95% CI = 9-13 IU/mL)	Frankenhuyszen et al. 1992
<i>B.t.k.</i> HD-1 strain (Thuricide 32 LV)	Spruce budworm (<i>Choristoneura fumiferana</i>) larvae (early 4 th instar) via dietary exposure	LC ₅₀ = 63 IU/mL diet (95% CI = 46-82 IU/mL)	Frankenhuyszen et al. 1992
<i>B.t.k.</i> (Thuricide 32 LV) (84 BIU/L)	Spruce budworm (<i>Choristoneura fumiferana</i>) exposed via diet for 14 days	LC ₅₀ = 160 IU/mL diet (95% CI = 139-183 IU/mL)	Frankenhuyszen and Fast 1989
<i>B.t.k.</i> (Thuricide 32 LV) (84 BIU/L)	Western spruce budworm (<i>Choristoneura occidentalis</i>) 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 (Beetles)			
<i>B.t.k.</i> (Dipel 4L) []	Convergent lady beetle (<i>Hippodamia convergens</i> 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 <i>B.t.k.</i> 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).			
<i>B.t.k.</i> CGA-237218	Ladybird beetles (<i>Coccinella septempunctata</i>), 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 <i>B.t.k.</i> formulation and oil blank.	Addison and Holmes 1995
Dipel 8AF (aqueous) as well as unformulated <i>B.t.k.</i>		No effects on Collembolan populations.	
Dermaptera (earwigs)			
<i>B.t.k.</i> (Dipel WP)	Striped earwig (<i>Labidura riparia</i>) 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)			
<i>B.t.k.</i> HD-1 (serovar 3a3b)	Laboratory bioassay in Mexican fruit fly (<i>Anastrepha ludens</i>).	Significant mortality from both pellet and supernatant preparations of <i>B.t.k.</i> in agar. Screening study using a variety of different <i>B.t.</i> strains to test for efficacy. Not directly useful for dose-response comparisons.	Robacker et al. 1996
Hemiptera (Bedbugs, aphids, cicadas)			
<i>B.t.k.</i> (Bactospeine WP) produced in the Netherlands	Spined soldier bug (<i>Podisus maculiventris</i>) (4 th instars and 7-day-old female adults) exposed to <i>B.t.k.</i> 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			
<i>B.t.k.</i> , Bactec Corp. 14.5 BIU per lb	Honey bees (<i>Apis mellifera</i>): 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.]
<i>B.t.k.</i> NOS	Honey bees	10-day LC 118 ug/bee (consumed)	MRID 435681-01 summarized but not referenced in U.S. EPA 1998
<i>B.t.k.</i> 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
<i>B.t.k.</i> (Commercial formulation containing 18,000 IU/mg)	Chinese praying mantis (<i>Tenodera aridifolia sinensis</i>) exposed via consumption of cabbage looper larvae that had consumed <i>B.t.k.</i> 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 (<i>Chrysoperla carnea</i>) 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
<i>B.t.k.</i> (Dipel 4L)	Common green lacewing (<i>Chrysopa carnea</i> 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
<i>B.t.k.</i> Biobit	Common green lacewing (<i>Chrysoperla carnea</i>), 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
<i>B.t.k.</i> CGA-237218	Green lacewing (<i>Chrysoperla carnea</i>), 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 <i>B.t.k.</i> Omitted Kirkland 1991, Nelson 1991b, and Palmer and Beavers 1993 studies on <i>B.t.</i> Abbott ABG-6305. This is <i>B.t.a.</i>			

Appendix 5: Toxicity of <i>B.t.k.</i> and <i>B.t.k.</i> 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 [<i>p</i> -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 limits.]	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 <i>B.t.k.</i> was higher than anticipated based on aqueous and dietary concentrations – it is unclear how this determination was made. No inflammation or necrosis.	Christensen 1990g
<i>B.t.k.</i> 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 40,000x expected environmental concentrations (EEC). Decreased mean body length and weight in exposed fish. No other signs of toxicity.	Christensen 1990i
<i>B.t.k.</i> 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 <i>B.t.k.</i> found in dead fish. Two fish has gill lesions from which <i>B.t.k.</i> could be cultured. The concentration in gills was less than the concentration in water.	Christensen 1991c
<i>B.t.k.</i> 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 <i>B.t.k.</i> and <i>B.t.k.</i> Formulations to Fish.			
Product	Species/Exposure	Observations	Reference
<i>B.t.k.</i> (wetable powder formulation manufactured in India)	Mosquito fish (<i>Gambusia affinis</i>) 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 $0, 5 \times 10^9, 1 \times 10^{10}, 1.5 \times 10^{10}, 2 \times 10^{10}$, 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
<i>B.t.k.</i>	Rainbow trout, 96 hour exposure	LC ₅₀ > 10 mg/L	Mayer and Ellersieck, 1986
<i>B.t.k.</i>	Bluegill sunfish, 96 hour exposure	LC ₅₀ = 95 mg/L	Mayer and Ellersieck, 1986
<i>B.t.k.</i> as unformulated product in Foray 48B	Koi carp (<i>Cyprinus carpio</i>) exposed to 1x or 10x ECC via food and water in experimental tanks for 32 days	Small quantities of bacteria unrelated to <i>B.t.</i> were recovered from various fish organs; bacteria occurred predominantly in the intestine; <i>B.t.</i> found intermittently; some of the <i>B.t.</i> strains isolated were not the strain applied to the tank; sublethal effects observed in the treated fish were independent of <i>B.t.</i> 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.
<i>B.t.k.</i> 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 evaluated.
Omitted Bellantoni et al. 1991a,d on Delta BT. Cannot identify strain.			

Appendix 6: Toxicity of <i>B.t.k.</i> and <i>B.t.k.</i> Formulations to Aquatic Invertebrates (sorted by specified group – phylum, order, or subclass – followed by studies on mixed populations).			
Cladocera			
Dipel, NOS	<i>Daphnia magna</i> , 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
<i>B.t.k.</i> CGA-237218 [Specified as containing 1.06×10 ¹¹ cfu/g equivalent to 1.06×10 ⁸ cfu/mg].	<i>Daphnia magna</i> , 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			
<i>B.t.k.</i> technical material	<i>Amphiascus minutus</i> (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 <i>B.t.k.</i> 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
<i>B.t.k.</i> 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 <i>B.t.k.</i> and <i>B.t.k.</i> Formulations to Aquatic Invertebrates (sorted by specified group – phylum, order, or subclass – followed by studies on mixed populations).			
Class Shrimp (Palaemonetes) (continued)			
<i>B.t.</i> 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			
<i>B.t.k.</i> (Dipel 64 AF)	Caddisfly (<i>Hydatophylas argus</i>) 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			
<i>B.t.k.</i> (Thuricide 32 LV containing 8.45 BIU/L)	Larvae of Simuliidae, 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 <i>Simulium vittatum</i> (black fly) in which only 6 adults emerged at 430 IU/mL; possible signs of toxicity were observed in <i>Prosimulium fascum/mixtum</i> (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*)

<p><i>B.t.k.</i> (Dipel 8AF with potency of 16.9 BIU/L)</p>	<p>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</p>	<p>No significant mortality in 11 species after 9 days; average mortality of 30% in stoneflies (<i>Taeniopteryx nivalis</i>) after 9 days.</p>	<p>Kreutzweiser et al. 1992</p>
<p><i>B.t.k.</i> (Dipel 8AF with potency of 16.9 BIU/L) About 0.00006 mg/BIU.</p>	<p>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.</p>	<p>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.</p>	<p>Kreutzweiser et al. 1992</p>
<p><i>B.t.k.</i> (Dipel 64AF)</p>	<p>caddisflies, mayflies, stoneflies (12 taxa) exposed to 10x label application</p>	<p>Only the stonefly (<i>Leuctra tenuis</i>) was reduced at 4 days after treatment</p>	<p>Kreutzweiser et al. 1993. Summarized in USDA 1995</p>

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*)

<i>B.t.k.</i> (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 <i>B.t.k.</i> 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 (<i>L. tenuis</i>) (~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
<i>B.t.k.</i>	50-5000 BIU/ha over streams.	No effect on benthic stream communities or insect emergence. Increased drift rates in mayfly (<i>Baetis sp</i>)	Richardson and Perrin 1994
<i>B.t.k.</i>	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, <i>B.t.a.</i>			