



**TM-BIOCONTROL:
A Preparation of Polyhedral Inclusion Bodies
of the Douglas Fir Tussock Moth (*Orgyia
pseudotsugata*) Nuclear Polyhedrosis Virus
Final Report**

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TABLE OF CONTENTS

ACRONYMS, ABBREVIATIONS, AND SYMBOLS	vii
COMMON UNIT CONVERSIONS AND ABBREVIATIONS	viii
CONVERSION OF SCIENTIFIC NOTATION	ix
EXECUTIVE SUMMARY	xi
1. INTRODUCTION	1-1
2. PROGRAM DESCRIPTION	2-1
2.1. DESCRIPTION AND COMMERCIAL FORMULATIONS	2-1
2.2. APPLICATION METHODS, RATES, AND MIXING	2-3
3. HUMAN HEALTH RISK ASSESSMENT	3-1
3.1. HAZARD IDENTIFICATION	3-1
3.1.1. Nuclear polyhedrosis viruses (NPVs) Including OpNPV	3-2
3.1.2. Effects Associated with the Douglas-fir Tussock Moth	3-2
3.1.3. Bacterial Contamination	3-3
3.1.4. Acute Systemic Toxic Effects	3-3
3.1.5. Effects on the Skin	3-3
3.1.6. Effects on the Eyes	3-4
3.1.7. Effects Associated with Inhalation/Pulmonary Exposures	3-4
3.1.8. Subchronic and Chronic Toxicity	3-5
3.1.9. Carcinogenicity and Mutagenicity	3-5
3.2. EXPOSURE ASSESSMENT	3-8
3.3. DOSE-RESPONSE ASSESSMENT	3-19
3.4. RISK CHARACTERIZATION	3-27

TABLE OF CONTENTS *(continued)*

4. ECOLOGICAL RISK ASSESSMENT 4-1

 4.1. HAZARD IDENTIFICATION 4-1

 4.1.2. Toxicity to Terrestrial Organisms 4-2

 4.1.3. Aquatic Organisms 4-5

 4.2. EXPOSURE ASSESSMENT 4-6

 4.3. DOSE-RESPONSE ASSESSMENT 4-15

 4.4. RISK CHARACTERIZATION 4-21

5. REFERENCES 5-1

6. GLOSSARY 6-1

7. SUBJECT INDEX 7-1

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

a.e.	acid equivalents
a.i.	active ingredient
A.U.	activity units
AEL	adverse-effect level
ACGIH	American Conference of Governmental Industrial Hygienists
AChE	acetylcholinesterase
ATSDR	Agency for Toxic Substances and Disease Registry
bw	body weight
CBI	confidential business information
CfNPV	<i>Choristoneura fumiferana</i> (spruce budworm) nuclear polyhedrosis virus
CFU	colony forming units
cm	centimeter
DF	dry flowable
d.f.	degrees of freedom
EC ₂₅	concentration causing 25% inhibition of a process
EC ₅₀	concentration causing 50% inhibition of a process
F	female
FS	Forest Service
g	gram
HQ	hazard quotient
kg	kilogram
L	liter
lb	pound
LC ₅₀	lethal concentration, 50% kill
LD ₅₀	lethal dose, 50% kill
LdNPV	<i>Lymantria dispar</i> (gypsy moth) nuclear polyhedrosis virus
LOAEL	lowest-observed-adverse-effect level
m	meter
M	male
MCS	multiple chemical sensitivity
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
mM	millimole
MNPV	multinucleocapsid nuclear polyhedrosis virus
MW	molecular weight
MOS	margin of safety
MSDS	material safety data sheet
NCI	National Cancer Institute

ACRONYMS, ABBREVIATIONS, AND SYMBOLS (*continued*)

NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NPV	nuclear polyhedrosis virus
NRC	National Research Council
OB	occlusion body
OpMNPV	<i>Orygia pseudotsugata</i> (Douglas-fir tussock moth) multinucleocapsid nuclear polyhedrosis virus
OpSNPV	<i>Orygia pseudotsugata</i> (Douglas-fir tussock moth) single nucleocapsid nuclear polyhedrosis virus
OPPTS	Office of Pesticide Planning and Toxic Substances
PIBs	polyhedral inclusion bodies
ppm	parts per million
RBC	red blood cells
RED	reregistration eligibility decision
RfD	reference dose
SNPV	single nucleocapsid nuclear polyhedrosis virus
TGAI	technical grade active ingredient
UF	uncertainty factor
U.S.	United States
U.S. EPA	U.S. Environmental Protection Agency
USDA	U.S. Department of Agriculture
>	greater than
≥	greater than or equal to
<	less than
≤	less than or equal to
=	equal to
≈	approximately equal to
~	approximately

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

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

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

CONVERSION OF SCIENTIFIC NOTATION

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

EXECUTIVE SUMMARY

INTRODUCTION

The USDA Forest Service uses TM-Biocontrol in the control of the Douglas-fir tussock moth (*Orgyia pseudotsugata*). TM-Biocontrol is a preparation of polyhedral inclusion bodies (PIBs) of the Douglas-fir tussock moth nuclear polyhedrosis virus (OpNPV). TM-Biocontrol was recently re-registered by the U.S. EPA along with Gypchek, a preparation of a related virus, Gypsy Moth (*Lymantria dispar*) NPV or LdNPV, which is used to control the Gypsy moth.

OpNPV is a naturally occurring virus that can substantially reduce Douglas-fir tussock moth populations. Like most other nuclear polyhedrosis viruses, OpNPV appears to be highly specific and does not infect or cause adverse effects in non-target species. This virus, like other nuclear polyhedrosis viruses, is enclosed within a crystal-like protein matrix. In infected cells, the virus forms polyhedral inclusion bodies (PIBs) in the nuclei of the host cell, which results in cell death and the release of PIBs to the environment. In naturally occurring NPV infections, the PIBs are usually ingested by the host larvae. In the alkaline pH of the larval gut, the PIBs are solubilized and virus is released, damaging various types of cells after replicating itself and eventually causing death in the larvae which in turn results in the release of additional viruses as well as insect parts to the environment. The persistence of OpNPV in the environment and the impact of TM-Biocontrol treatment on the prevalence of OpNPV in the environment is well characterized. In general, treating a Douglas-fir tussock moth infestation with TM-Biocontrol will result in lower levels of OpNPV in the environment, compared with levels that would be released after the collapse of untreated infestations. With or without TM-Biocontrol treatment, OpNPV persists in soils for several years.

The risk assessment of OpNPV is qualitatively different in some ways from risk assessments of chemical agents. Most nuclear polyhedrosis viruses (NPV) including OpNPV are highly host specific. In other words, a particular NPV is typically infective and pathogenic in a single or very small number of species. OpNPV is highly infective and pathogenic to the Douglas-fir tussock moth and is also pathogenic to the white-marked tussock moth, *Orgyia leucostigma*. In these species, the virus causes a well-characterized effect for which the most meaningful measure of exposure is clearly the number of active polyhedral inclusion bodies. For other species, including humans and wildlife species, it is not clear that PIBs are a meaningful measure of exposure because the NPV does not appear to have any effect on these species. Instead, the available information suggests that most adverse effects in non-target species, particularly humans, are associated with exposure to the insect parts in the commercial formulation.

PROGRAM DESCRIPTION

TM-Biocontrol was developed by the USDA Forest Service as a replacement for DDT, which was used for the control of the Douglas-fir tussock moth. TM-Biocontrol is produced by the *in vivo* culture of infected moth larvae. In the late 1980s, the purity of TM-Biocontrol was reported as 3.5% (w/w); however, more recent formulations have an average purity of 11.6% (w/w). This increase in average purity suggests that recent formulations of TM-Biocontrol are more refined than earlier formulations. By comparison, Gypchek contains 20% (w/w) LdNPV. In addition to

PIBs and larval parts, TM-Biocontrol contains bacteria that are endogenous to the tussock moth. As with any biological preparation, there is a potential for contamination with pathogenic bacteria. Consequently, during manufacture, the Forest Service conducts several assays to ensure that bacterial pathogens do not contaminate TM-Biocontrol.

Application rates or other measures of exposure to TM-Biocontrol can be expressed in various units, the most common of which are weight of formulation, weight of the virus PIBs, counts of the polyhedral inclusion bodies (PIBs), or activity units (A.U.) based on an *in vivo* bioassay of the formulation using the Douglas-fir tussock moth. The most reliable measure of biological activity to the target insect is activity units. In the re-registration of TM-Biocontrol, however, the U.S. EPA uses PIBs rather than activity units. For both ground and aerial applications, the recommended application rate is 0.4 oz or 11 grams of TM-Biocontrol per acre. Prior to application, the TM-Biocontrol formulation is mixed with water, molasses, and a whitening agent. The whitening agent is intended to protect the PIBs from inactivation due to exposure to sunlight.

HUMAN HEALTH EFFECTS

Hazard Identification

The hazard identification process for the human health risk assessment involves determining what endpoints an agent is likely to induce in humans. This assessment is based on human data as well as data on experimental mammals. The topics typically covered in a hazard identification include acute, subchronic, and chronic systemic toxic effects as well as the assessment of the potential for certain endpoints of particular concern, including reproductive and teratogenic effects, carcinogenicity, and irritant effects. For biological control agents, additional endpoints of particular concern are infectivity (the ability to survive in an organism) and pathogenicity (the ability to grow in and damage an organism).

For OpNPV, another area of concern is the potential impact of insect parts of the Douglas-fir tussock moth, which are known irritants and allergens in humans. Several moth larvae, including those of the Douglas-fir tussock moth, have hairs that can cause skin, eye, and respiratory irritation in humans. Studies of human populations exposed to Douglas-fir tussock moth infestations indicate that the prevalence of these effects in humans may range from approximately 25% to 75%.

Most of the available mammalian toxicity data on TM-Biocontrol was generated in the mid-1970s as part of the registration process and involved only assays for acute toxicity and infectivity/pathogenicity. Most of these studies involved relatively small numbers of animals and assay for only a limited number of effects. Single oral (gavage) doses of 3160 and 10,000 mg/kg caused no mortality, overt signs of toxicity, or gross pathological changes in rats over a 28-day observation period. Injections of 500 mg/kg of TM-Biocontrol into the abdomen of mice were fatal within 4 hours of dosing. No effects were seen after injections of 5 or 50 mg/kg. The relatively rapid death of the mice suggests that the mortality is not attributable to infectious bacterial contamination.

TM-Biocontrol is known to cause skin, eye, and respiratory tract irritation. The available human data regarding the effects of exposure to Douglas-fir tussock moth larvae suggest that the irritant effects are probably due to the occurrence of insect parts in the TM-Biocontrol formulation. In a standard assay for eye irritation—0.1 g or 100 mg in the eyes of rabbits—moderate eye irritation was noted over a 28 day post-exposure period. At a much lower dose, 3.0 mg per eye, slight and transient conjunctival irritation was noted with full recovery after 48 hours. A comparison of exposure studies involving undiluted formulations of TM-Biocontrol and Gypchek indicates that of the two biological control agents, TM-Biocontrol is a stronger eye irritant.

Exposure Assessment

In the re-registration of both OpNPV and LdNPV, the U.S. EPA determined that formal exposure assessments for the general public and workers were not required because of the lack of any apparent hazard of systemic toxic effects and because the use of TM-Biocontrol will not substantially increase ambient levels of both NPV and insect larval parts. Based on calculations presented in this risk assessment, it appears that treatment of a severe Douglas-fir tussock moth infestation with TM-Biocontrol would increase the environmental levels of NPV by about 3% or less. In addition, the use of TM-Biocontrol to prevent a severe infestation would reduce eventual exposures to both OpNPV as well as insect larvae.

Dose-Response Assessment

As with the exposure assessment, there is no basis for conducting a dose-response assessment for systemic toxic effects because no systemic toxic effects can be qualitatively identified for plausible routes of exposure (i.e., oral, dermal, or inhalation). Nonetheless, TM-Biocontrol may cause skin and eye irritation and these endpoints are of concern at least for occupational exposures.

In the re-registration of TM-Biocontrol, the U.S. EPA used data on Gypchek to assess some of the possible risks of exposure to TM-Biocontrol. Based on an eye irritation study using Gypchek at twice the concentration of a typical field application solution (2X), the U.S. EPA judged that both Gypchek and TM-Biocontrol will not cause eye irritation at field dilutions (1X). Nevertheless, the available data on technical grade formulations (i.e., undiluted formulations) suggest that of the two biological control agents, TM-Biocontrol is a somewhat stronger eye irritant.

Risk Characterization

There is no basis for asserting that workers are subject to any risk of systemic adverse effects in the use of TM-Biocontrol. Nonetheless, workers involved in the mixing of TM-Biocontrol will be exposed to the undiluted formulation and there is a potential for skin, eye, and perhaps respiratory tract irritation. Even in the application of field dilutions of TM-Biocontrol, it would be prudent for workers to take reasonable measures and use personal protective equipment to limit the potential for introducing either undiluted formulation or field dilutions of TM-Biocontrol into the eyes.

Infestations of the Douglas-fir tussock moth tend to occur in relatively remote areas and members

of the general public are not likely to be exposed to TM-Biocontrol in the treatment of such infestations. Even if members of the general public were exposed to a spray of TM-Biocontrol, the primary concern would be the insect parts in the formulation. Because applications of TM-Biocontrol will not substantially increase ambient exposures to either OpNPV or the insect parts and because the use of TM-Biocontrol will, over the longer term, reduce exposures to OpNPV and the Douglas-fir tussock moth, the use of TM-Biocontrol may be judged as beneficial rather than potentially detrimental to members of the general public.

ECOLOGICAL EFFECTS

Hazard Identification

As with the information used in the human health risk assessment, most of the ecological studies on TM-Biocontrol were conducted in the mid-1970s as part of the registration process and involved few animals, short-term exposures, and assays for relatively gross effects. Also as in the human health risk assessment, there is no indication that OpNPV is infective or pathogenic and there is little indication that even high dose levels of TM-Biocontrol will cause adverse effects in vertebrates. One possible exception involves a 5-day oral bioassay using three female mallard ducks in which muscular weakness was apparent in one of the animals after the first exposure and persisted throughout a 40-day observation period. This study was obviously a preliminary screen for subchronic toxicity. That an effect was seen in one animal shortly after the first dose and that the effect persisted for 40 days is not consistent with the other information on the effects of TM-Biocontrol. The most likely explanation for the observed effect is that the animal was injured incidentally by dosing—gavage with glass tubing. Nonetheless, the best way to clarify this would be to conduct a standard subchronic feeding study in mallards.

Based on a substantial amount of information on NPV in general and OpNPV specifically, there is no indication that the virus in TM-Biocontrol is likely to cause adverse effects in non-target insects or aquatic species. No phytotoxicity studies on OpNPV were encountered but several field trails using OpNPV have failed to note any indication of phytotoxicity attributable to OpNPV.

Exposure and Dose-Response Assessments

As with the human health risk assessment, a formal exposure assessment for TM-Biocontrol is not justified because the application of TM-Biocontrol in areas infested by the Douglas-fir tussock moth will not substantially increase exposure to either OpNPV or the larval parts. In fact, treatment of a Douglas-fir tussock moth infestation with TM-Biocontrol is mostly likely to reduce exposure to both the larval parts and the virus. Also similar to the human health risk assessment, the hazard identification for TM-Biocontrol is essentially negative (i.e., there is little basis for asserting that TM-Biocontrol poses any risk to non-target species). Therefore, a dose-response assessment is not warranted.

Risk Characterization

As in the human health risk assessment, there are three agents that could be of concern in TM-Biocontrol: the virus, the insect parts, and incidental bacterial contamination. There is no indication that OpNPV is pathogenic to species other than the Douglas-fir tussock moth, the western tussock moth, the rusty tussock moth, and the white-marked tussock moth. To the contrary, experience with OpNPV as well as other related NPV indicate that these viruses have a very narrow host range and do not infect non-target species. The Forest Service takes reasonable measures to control for possible incidental contamination of TM-Biocontrol by pathogenic bacteria. The formulation does contain bacteria that are endogenous to the Douglas-fir tussock moth and these bacteria might possibly account for some of the effects observed in animals. Nonetheless, the larvae of the Douglas-fir tussock moth are known to contain hairs that cause irritant and perhaps allergic reactions. Thus, the larval parts in TM-Biocontrol are the most likely cause of the few effects observed in TM-Biocontrol studies.

As is also true for the human health risk assessment, the over-riding consideration in the risk characterization for non-target species is that the use of TM-Biocontrol will reduce rather than increase exposure to the Douglas-fir tussock moth and OpNPV.

1. INTRODUCTION

The USDA Forest Service uses TM-Biocontrol in the control of the Douglas-fir tussock moth (*Orgyia pseudotsugata*). TM-Biocontrol is a preparation of polyhedral inclusion bodies (PIBs) of the Douglas-fir tussock moth nuclear polyhedrosis virus (NPV). Based on the recent re-registration eligibility decision (RED, U.S. EPA 1996) and a few more recent studies not cited in the RED, the present document provides risk assessments for human health effects and ecological effects of OpNPV to support an assessment of the environmental consequences of using TM-Biocontrol in Forest Service programs. In the re-registration process, the U.S. EPA (1996) combined data from the Tussock Moth NPV (OpNPV) and a related virus, Gypsy Moth (*Lymantria dispar*) NPV (LdNPV). Thus, while focused on OpNPV, this document also covers some information on LdNPV and other related NPVs.

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

Nonetheless, this risk assessment of OpNPV is qualitatively different in some ways from risk assessments of chemical agents. Because NPVs are biological organisms rather than chemicals, many standard physical and chemical properties used to characterize chemical compounds and estimate certain exposure parameters (e.g., SERA 1998) simply do not apply to OpNPV or other NPVs. More significant is the fact that most NPVs including OpNPV are highly host specific. OpNPV is pathogenic to the Douglas-fir tussock moth as well as three related species, the white-marked tussock moth (*Orgyia leucostigma*), the western tussock moth (*Orgyia cana*), and the rusty tussock moth (*Orgyia antiqua*) (Hughes 1976). In these species, OpNPV produces a well-characterized effect for which the most meaningful exposure metameter is clearly the number of active polyhedral inclusion bodies (PIBs). For other species, including humans, PIBs are a less meaningful measure of exposure because OpNPV does not appear to affect non-target species. Instead, the available information suggests that most adverse effects in non-target species associated with exposure to TM-Biocontrol are likely to be associated with insect parts in the commercial formulation.

This is a technical support document and it addresses some specialized technical areas. Nevertheless, an effort has been made to ensure that the document can be understood by individuals who do not have specialized training in the chemical and biological sciences. Certain technical concepts, methods, and terms common to most risk assessments are described in a separate document (SERA 1998). In addition, technical terms commonly used in this document are defined in the glossary (chapter 6).

The human health and ecological risk assessments presented in this document are not, and are not intended to be, comprehensive summaries of all of the available information. Most of the literature on OpNPV is summarized in the RED (U.S. EPA 1996). Additional information on the development and use of TM-Biocontrol was published by Martignoni (1999). Most of the mammalian toxicology studies and some ecotoxicology and environmental fate studies are unpublished reports submitted to the U.S. EPA as part of the registration or re-registration of OpNPV. Full text copies of all studies submitted to the U.S. EPA were provided by the USDA Forest Service. These studies were reviewed and are discussed in this document. In addition, three supplemental studies sponsored by the Forest Service were also reviewed (David 1989a,b,c).

2. PROGRAM DESCRIPTION

2.1. DESCRIPTION AND COMMERCIAL FORMULATION

TM-Biocontrol is a preparation of polyhedral inclusion bodies (PIBs) of the Douglas-fir tussock moth (*Orgyia pseudotsugata*) multinucleocapsid nuclear polyhedrosis virus (OpMNPV). TM-Biocontrol was developed as a replacement for DDT, which previously was used for the control of the Douglas-fir tussock moth (Crouch and Perkins 1968). The primary host for this species of tussock moth is the Douglas-fir (*Pseudotsuga menziesii*), although true fir (*Abies spp.*) are also be attacked (Ross and Arrand 1976, Shepherd 1980). Periodic infestations by the Douglas-fir tussock moth can result in substantial defoliation in Douglas-fir and other true-fir forests in the western United States and western Canada (Martignoni 1999).

The USDA Forest Service holds the registration for TM-Biocontrol, and much of the early research on the characterization of NPV pathogens in the Douglas-fir tussock moth was conducted as a collaborative effort between the Forest Service and Oregon State University (Rohrmann 1977, Rohrmann et al. 1978a,b). OpNPV was first noted in 1947 by Forest Service researchers as the result of a collapse of a Douglas-fir tussock moth population in an area that was not treated with DDT (Evenden and Jost 1947). During a severe outbreak of the Douglas-fir tussock moth in 1964, the Forest Service began an active research program to develop OpNPV preparations that could be applied to infested areas. After several field trials, the Forest Service applied to the U.S. EPA for product registration in 1976 (Martignoni 1999). On August 11, 1976, the U.S. EPA approved the U.S. Forest Services' application for registration of PIBs of OpNPV as a viral insecticide for controlling the Douglas-fir tussock moth and issued a reregistration eligibility decision for the product in 1996 (U.S. EPA 1996).

TM-Biocontrol is produced by the *in vivo* culture of infected moth larvae. Details of this process are included in the registration for TM-Biocontrol (Martignoni 1978). Larvae are reared at the production facility and are inoculated with a base or primary OpNPV culture. OpNPV is taken from these larvae and used as a secondary inoculum on additional larvae. Using this process, a total of 2.15×10^{12} PIBs can be obtained from an initial inoculum of 10^9 PIBs. The secondary inoculum is then fed to fifth instar larvae. The larvae are cultured at 25°C and harvested 8-14 days after exposure. Harvesting consists of isolating PIBs from the infected larvae. The larvae are blended and passed through a series of screens. The resulting preparation is then lyophilized and milled to a fine powder. Using this process, the average yield is 6.7×10^8 PIBs per larva (Martignoni 1978).

In the late 1980s, the purity of TM-Biocontrol was reported as 3.5% (w/w) (USDA 1988, section 1.2). In this context, purity presumably refers to the percent weight of the PIBs relative to the total weight of the formulations. More recently, an average purity of 11.6% (w/w) or 21.9 billion PIBs per gram was reported (USDA 1998, Confidential Statement of Formulation). Thus, it appears that more recent formulations of TM-Biocontrol are more refined than earlier formulations. By comparison, Gypchek, the NPV for the control of the Gypsy moth, contains 20% (w/w) LdNPV (USDA 1995).

The primary contaminants in OpNPV preparations include parts of the larvae in which the virus was cultured (USDA 1976a, p. 32) and nonpathogenic coliform bacteria (Tucker 1966).

2.2. APPLICATION METHODS, RATES, AND MIXING

Application rates or other measures of exposure to TM-Biocontrol can be expressed in various units, the most common of which are weight of formulation, weight of the virus PIBs, counts of the polyhedral inclusion bodies (PIBs), or activity units (A.U.) based on an *in vivo* bioassay of the formulation using the Douglas-fir tussock moth. The most reliable measure of biological activity to the target insect is activity units. These are calculated using an *in vivo* oral bioassay as detailed by Martignoni and Iwai (1977). Martignoni (1999, p. 34) indicates that 1×10^{11} PIBs/acre was equivalent to 1.1×10^9 activity units/acre for one batch of TM-Biocontrol used in the late 1970s. Using these values, the relationship of PIBs to activity units is 0.91×10^2 or 91 PIBs/activity unit [1×10^{11} PIBs/acre \div 1.1×10^9 activity units/acre].

In the re-registration of TM-Biocontrol, however, the U.S. EPA uses PIBs rather than activity units. For both ground and aerial applications, the recommended application rate is 0.4 oz or 11 g of TM-Biocontrol per acre (USDA 1999a). According to the most recent product label (USDA 1999a), current formulations of TM-Biocontrol contain at least 621.7 billion [621.7×10^9 or 6.217×10^{11}] PIBs/gram. Thus, the application rate in terms of PIBs is about 6.8×10^{12} PIBs/acre [$11 \text{ g TM-Biocontrol/acre} \times 6.217 \times 10^{11} \text{ PIBs/g TM-Biocontrol} = 6.8387 \times 10^{12} \text{ PIBs/acre}$]. Prior to application, TM-Biocontrol is mixed with water, molasses, and a whitening agent. Presumably, the whitening agent is intended to protect the PIBs from inactivation due to exposure to sunlight. The pH of the solution is maintained between 6.0 and 7.2 with the addition of sodium hydroxide.

In aerial applications, appropriate amounts of TM-Biocontrol are sprayed in 2 gallons of the field mixture per acre. Boom and nozzle systems or atomizers are used to produce spray droplets of 50-100 microns volume median diameter. TM-Biocontrol is never added directly to the aircraft hopper. Ground applications may involve the use of hydraulic sprayers at a rate of 100-200 gallons of water per acre. Spray equipment mounted on tractors or trucks is used to broadcast the agent onto vegetation that might be consumed by the moth larvae. When individual trees are treated, they are sprayed to runoff, typically about 15-20 gallons for a large Douglas-fir tree.

3. HUMAN HEALTH RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION

3.1.1. Nuclear Polyhedrosis Viruses (NPV) Including OpNPV.

General reviews on the effects of nuclear polyhedrosis viruses indicate that these organisms, as a class, are highly host specific and do not infect or cause other adverse effects in non-target species, including mammals, birds, fish, plants, microorganisms, or cell lines from vertebrates and non-arthropod invertebrates (Döller 1985, Groner 1986). Several recent studies on OpNPV focus on characterizing the genetic structure, virion associated proteins, or the mechanism of action of OpNPV and related viruses at the molecular level (Ahrens et al. 1997, Birnbaum et al. 1994, Kogan and Blissard 1994, Lu et al. 1996, Russell and Rohrmann 1997, Russell et al. 1997, Wu et al. 1993). Many of the different NPVs—including those isolated from the Douglas-fir tussock moth, the light brown apple moth (*Epiphyas postvittana*), and the tea moth (*Buzura suppressaria*)—have similar genetic structures (Cowan et al. 1994, Hu et al. 1998, Hyink et al. 1998).

OpNPV is a naturally occurring virus that can substantially reduce populations of the Douglas-fir tussock moth (Dahlsten and Thomas 1969). This virus, like other nuclear polyhedrosis viruses, is a rod-shaped nucleocapsid enclosed within a protein envelope. In infected cells, the viruses form polyhedral inclusion bodies (PIBs) in the nuclei of the host cell, which results in cell death and the release of PIBs to the environment. In naturally occurring NPV infections, the PIBs are typically ingested by the host larvae. In the alkaline pH of the larval gut, the PIBs are solubilized and release the virions. The nucleocapsids penetrate and replicate in the nuclei of mid-gut cells, eventually causing cell death and the release of additional virions into the insect that may infect other cells in the mid-gut or other cells including those in fat bodies, the epidermis, tracheal epithelium, hemocytes, and silk glands. When a sufficient number of vital cells are damaged, the insect dies (Martignoni 1999). Time to death is usually 1-2 weeks after initial exposure. When the insect dies, high numbers of virus polyhedra are released into the environment and the disease propagates to other Douglas-fir tussock moths. After the collapse of the moth population, OpNPV may persist in soil for several years (Thompson 1978a).

OpNPV is a member of the Baculoviridae that includes both nucleopolyhedroviruses, such as OpNPV and LdNPV, as well as granuloviruses (Döller 1985). Both budded viruses and occluded viruses are produced by baculoviruses. The budded viruses participate in cell to cell spreading of the infection, and the occluded viruses participate in the spread of the infection among individual insects in a population (Russell and Rohrmann 1997, Theilmann et al. 1996). In late stages of infections, the Baculoviruses pack virus particles or occluded viruses into occlusion bodies that have a polyhedral or granular shape. Baculoviruses have been isolated only from arthropods and are characterized by a very limited host range (Chou et al. 1996).

There are two general types of NPV, uninucleocapsid (SNPV) and multinucleocapsid (MNPV). Both types of virus, OpSNPV and OpMNPV, as well as a cytoplasmic polyhedrosis virus can infect the Douglas-fir tussock moth (Hughes 1976, Martignoni 1999). TM-Biocontrol contains only the multinucleocapsid form of the virus, OpMNPV, and a monoclonal antibody assay was developed to distinguish OpMNPV from OpSNPV (Quant et al. 1984). Virus particles with an abnormal appearance were observed in Douglas-fir tussock moths infected with OpSNPV but not with OpMNPV (Hughes 1976).

Naturally occurring levels of OpNPV in the environment associated with the collapse of Douglas-fir tussock moth populations are much greater than levels associated with the application of TM-Biocontrol to control Douglas-fir tussock moth populations. Each target larva can produce 2% to 20% of the PIBs in the original acre treatment at an application rate of 5×10^{10} PIBs/acre (Tucker 1966).

The persistence of OpNPV in the environment as well as the impact of TM-Biocontrol treatment on the prevalence of OpNPV in the environment is well characterized (Thompson 1975, Thompson and Scott 1979). In this series of studies, OpNPV was assayed in three areas with Douglas-fir tussock moth infestations. Two of the areas were treated with OpNPV during infestations at rates of 10^{12} PIBs/acre and 10^{11} PIBs/acre. The third area served as an untreated control. The levels of OpNPV in the top 1 and 5 cm of soil was greatest in the untreated control plot and inversely related to the application rate in the treated plots (Thompson 1975, Tables 2 and 3, pp. 56-57; Thompson and Scott 1979, Table 2, p. 61). The higher levels of OpNPV in the untreated plot was attributed to the greater numbers of larvae surviving in the untreated plot which in turn resulted in greater amounts of endogenous OpNPV being released when the larvae on the untreated plot became infected with endogenous OpNPV and died in the last two instars.

3.1.2. Effects Associated with the Douglas-fir Tussock Moth

The Douglas-fir tussock moth overwinters in the egg stage, hatching in the spring. The females are flightless and lay their eggs on the cocoons after emergence. The larvae, which are light and have many fine hairs, spin fine threads from which they hang and are dispersed by wind. Larvae may go through four or five molts, with most 6th stage instar larvae being female (Page and Lyon 1973). The larvae feed extensively on vegetation until the end of June or mid-July in the northern regions (Ross and Arrand 1976). Pupation lasts about 10-14 days, whereupon the adults emerge, mate, and the females deposit eggs. The eggs, larvae, and pupae are subject to various parasites but the most important naturally occurring control agent is OpNPV.

A number of moth larvae, including those of the Douglas-fir tussock moth, have hairs that can cause skin, eye, and respiratory tract irritation in humans. As reviewed by USDA (1995), severe Gypsy moth infestations may be associated with 20% to 30% of the incidences of skin rashes that are sufficiently severe to cause members of the general public to seek medical attention. Similar incidences, about 25%, of severe skin irritation were associated with

infestations of the mulberry tussock moth in China (De-Long 1981). As with the gypsy moth, the irritant and inflammatory effects of mulberry tussock moth hairs in humans is at least partially attributable to the occurrence of histamine in the moth hair.

During the summer of 1973, a severe outbreak of the Douglas-fir tussock moth occurred in Oregon and Washington and human health effects were observed in two studies (Perlman et al. 1976, Press et al. 1977). In both studies, the effects noted in exposed humans included signs of dermal, ocular, and respiratory irritation.

Perlman et al. (1976) examined 227 workers in whom the primary signs and symptoms of exposure to the Douglas-fir tussock moth included irritation to the eyes, skin and respiratory tract. Occupational groups, in order of subjective estimates of increasing exposures to the larvae, included lumber mill workers, forestry workers, and loggers with response rates of 41%, 44%, and 83%, respectively, compared with a response rate of 22% in a group of presumably unexposed workers. Defining extra risk, P , using Abbott's formula (Finney 1971),

$$P = \frac{P_e - P_c}{1 - P_c} \quad (1)$$

where P_c is the response in the control group and P_e is the response in the exposed group, the extra risks for mill workers, forestry workers, and loggers was 24%, 28%, and 78%. For mill workers and loggers, these rates are similar to the incidences of similar effects in the general public from severe infestations of the mulberry tussock moth and gypsy moth, as summarized in the previous paragraph.

For loggers, the incidence of 78% is substantially higher than the other two worker groups reported by Perlman et al. (1976) or the rates in the general public from severe infestations of the mulberry tussock moth and gypsy moth. While exposure metameters were not quantified, the most severe exposures were characterized as "*almost a rain of toxic and allergenic fall-out*" (Perlman et al. 1976, p. 303), presumably applying to loggers. This higher response rate in loggers may be a reflection of the severe exposure conditions rather than an indication that the Douglas-fir tussock moth is substantially more potent an irritant than either the Gypsy moth or the mulberry tussock moth. Conversely, as detailed in section 3.3, TM-Biocontrol appears to be a somewhat more potent irritant than Gypchek, the formulation of LdNPV that is made from the culture of Gypsy moth larvae, and this would be consistent with the possibility that the larval hairs of the Douglas-fir tussock moth are more potent irritants than those of the Gypsy moth.

The study by Press et al. (1977) reports data on the same group of workers as described by Perlman et al. (1976) as well as 428 individuals who were sent questionnaires concerning symptoms and levels of exposure to the Douglas-fir tussock moth. In the exposed workers, the major reported effects included irritation to the skin, eyes, and respiratory tract. In the most

severe cases, the effects were characterized as *respiratory difficulty*. Press et al. (1977) report incidences of irritant effects broken down by individuals with a prior history of allergies and individuals with no prior history of allergies. While individuals with a history of allergy generally had higher incidences of effects in all worker groups, a substantial proportion of loggers with no history of allergies complained of skin rashes or welts, suggesting that the insect parts may contain both primary irritants as well as allergenic materials.

3.1.3. Bacterial Contamination.

As indicated in section 2.1, some early preparations of OpNPV were contaminated with non-pathogenic coliform bacteria (Tucker 1966). Bacterial contamination occurs because TM-Biocontrol is produced by the culture of insect larvae, which contain endogenous bacteria. As detailed in a 1988 product registration package (USDA 1988, section 2.1.4), several different assays are conducted to monitor contamination by bacterial pathogens, including anaerobic agar cultures for anaerobic and micro-aerophilic bacteria, trypticase soy agar cultures for spores of aerobic bacteria, assays for coliform bacteria and typhoid, paratyphoid, and dysentery bacilli, as well as assays for human pathogenic Gram-negative bacteria (*Shigella* and *Salmonella*). Thus, while the Forest Service conducts assays for the occurrence of pathogenic bacteria, the contamination of TM-Biocontrol with non-pathogenic bacteria is inevitable.

The primary significance of the bacterial contamination of TM-Biocontrol pertains to the interpretation of studies that involve the injection of TM-Biocontrol. When injected into the body cavity (i.e., intraperitoneal injection), bacterial contamination along with general toxic or allergic responses to foreign bodies could cause effects that would have little relevance to the assessment of oral, dermal, or inhalation exposures.

3.1.4. Acute Systemic Toxic Effects

The acute oral toxicity of a OpNPV formulation was assayed in male and female albino rats, five per sex, after single gavage doses of 3160 and 10,000 mg/kg in methylcellulose (Weir 1967a). No deaths or overt signs of toxicity were noted over a 28-day post-exposure observation period, and necropsy revealed no gross pathological changes at terminal sacrifice.

The acute toxicity of OpNPV was also assayed after acute intraperitoneal injections (injections into the abdomen) to mice at doses of 0.1, 1.0, or 10 mg per animal (Lilja 1980). Assuming a 20g or 0.02 kg body weight, these doses expressed per animal correspond to doses of 5, 50, or 500 mg/kg body weight (bw). Significant mortality was noted at a dose 500 mg/kg bw, with all mice dying within 4 hours. No mortality or signs of toxicity, however, were noted at the two lower doses over a 21-day observation period and no indication of toxicity was noted at necropsy.

The apparently higher toxic potency of TM-Biocontrol after intraperitoneal injection relative to oral exposure is probably related to a general foreign body response. The relatively rapid death of the mice suggests that the mortality is not attributable to infectious bacterial contamination.

In an earlier study (Olitzky 1971), mortality was noted in only one of 24 mice after the intraperitoneal injection of an OpNPV formulation at a dose of 0.5 mL/20 g animals. Assuming a density of 1 g/mL TM-Biocontrol, a dose of 0.5 mL/20 g animal would correspond to a dose of 25,000 mg/kg bw [500 mg/0.02 kg]. This study does not specify the density of the OpNPV formulation or the amount of the formulation in the injected material. Thus, it is unclear if this study is directly comparable to the above study by Lilja (1980).

3.1.5. Effects on the Skin

Mathias (1981) studied a small group of workers involved in the application of OpNPV. The exposed group consisted of 13 workers and supervisory staff involved in the collection of Douglas-fir tussock moth larvae and the spraying of OpNPV. Skin rashes consistent with contact dermatitis from the larvae were noted in three of the 13 workers. Respiratory tract symptoms, not otherwise specified, were not associated with spraying of NPV. Subsequent analysis of blood samples from these workers for antibodies to OpNPV did not suggest any immunological response compared with blood samples from unexposed workers (Kaupp 1982).

OpNPV was assayed in a standard primary skin irritation study after application to the intact and abraded skin of rabbits at a dose of 0.5 g TGAI (Weir 1967b). At 24 hours, the only effect noted was slight erythema on the rabbits whose skin was abraded prior to application of the test material.

Tucker (1966) reported slight erythema and edema as well as slight necrosis of the stratum germinativum in rabbits after exposure to a 10% w/v solution of OpNPV. A bioassay for primary skin irritation was conducted on the abraded and intact skin of six albino rabbits using a patch test with a 24-hour exposure period and 72-hour observation period (Weir 1968). Very slight erythema was noted in some of the animals after 24 hours but not at 72 hours, and the OpNPV formulation was classified as mildly irritating. In a skin sensitization assay, an OpNPV formulation was also found to be inactive in guinea pigs (Weir 1967c).

David (1989a) assayed the dermal toxicity of a TM-Biocontrol formulation “with a standard aerobic plate count of 2.3×10^8 CFU/g”. In this context, *CFU* presumably refers to colony forming units and probably involved an assay of bacterial contaminants and not viable virus. Each of five male and five female rabbits were exposed to a single dermal dose of 2 g/kg and observed for 14 days. The test material was covered for 24 hours after application. Moderate dermal irritation was noted in all animals on day 1. In male rabbits, irritation was noted on day 2 in four of five animals, which progressed to desquamation (shedding or peeling of the skin) on day 3. Slight desquamation was noted in one of five males on day 7. In female rabbits, irritation was also noted on day 2 (two of five) and desquamation in one of five animals on day 3. No mortality, changes in body weight, or signs of systemic toxicity were noted in any animals over the 14-day observation period, and no treatment related lesions were noted at gross necropsy.

3.1.6. Effects on Eyes.

Ocular irritation of an OpNPV formulation was assayed in nine albino rabbits after the instillation of 3.0 mg into the left eye (Weir 1967a). The right eye served as a control. In six of the nine rabbits, the exposed eye was irrigated after instillation. The time to irrigation is not specified in the study. In the irrigated eyes, no irritation was noted. In two of the three non-irrigated eyes, conjunctival irritation was observed and characterized as “*slight discharge and/or slight redness*”. These changes subsided after 48 hours, and fluorescein examination after 7 days revealed no evidence of corneal damage.

Another acute eye irritation study in rabbits was conducted by David (1989b) using the same formulation as described in section 3.1.4. The left eye of each of six rabbits was treated with 0.1 g of the test material, with the right eye of each animal serving as an untreated control. The eyes were examined at 1 hour and 1, 2, 3, 9, and 21 days post-treatment. At 1 hour, conjunctival irritation and swelling was observed in all animals but no corneal irritation was observed in any animals. Scattered corneal opacity was observed in all animals by day 2 after treatment. By day 21, conjunctival irritation was apparent in four of six animals and corneal damage ranging from scattered translucent areas (score= 1) to opalescent area (score= 3) was observed in four of six animals (Table 2, pp. 14-19, David 1989b). Using a Draize scoring system, the test material was classified as *Moderately Irritating*.

Subsequent to the RED (U.S. EPA 1996), the Forest Service funded two studies on the ocular irritation of Gypchek, the commercial formulation of LdNPV. One study used the commercial formulation (Kuhn 1997a) and the other study used an aqueous solution at twice the anticipated field concentration (Kuhn 1997b). Both studies identify the test material as a 3.65×10^{10} PIBs/g LdNPV preparation [Lot GR-14A], a wettable powder. The study by Kuhn (1997a) characterizes the applied material as a “*Gypchek TGAI*”, presumably referring to technical grade active ingredient, and indicating the raw technical material (i.e., the mixture of virus, insect parts and other ingredients). The study by Kuhn (1997b) characterizes the applied material as a “*Gypchek Solution 2X*”, presumably indicating that the test solution was diluted to a concentration that is twice that used in field applications. Kuhn (1997b) does not specify the actual concentration of the test solution. In a letter of clarification to the U.S. EPA, Kuhn (1997c) indicates that the 2X solution was a concentration of 2.92 mg technical product/mL. This dose is characterized as twice the field concentration based on a letter from Podgwaite (1996) indicating that the batch of Gypchek tested by Kuhn (1997a,b) would be diluted to 2×10^{11} PIBs/gallon and that this would correspond to 1.45 mg/mL.

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

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

3.1.7. Effects Associated with Inhalation/Pulmonary Exposures

The acute inhalation toxicity of OpNPV dust was assayed in rats using head-only exposure at concentrations ranging from 0.0049 to 0.79 mg/L (Thornett 1975). The large range of values represents only a single exposure group and reflects the variability in the measures of concentrations that were achieved in the exposure chamber. Five males and five females were exposed for 70 minutes, with additional groups of five males and five female rats serving as controls. No signs of toxicity were noted during or after exposure over a 2-week observation period.

Blood and lung tissue samples from the study by Thornett (1975) were sent to the Forest Service and were analyzed by Martignoni (Martignoni 1976, Martignoni and Iwai 1980). Clearance of the virus from the lungs followed a bi-exponential pattern, with the first phase presumably associated with bronchial or ciliary clearance and the second phase associated with alveolar or macrophage clearance. All viable viruses were cleared from the lungs after 168 hours. Analyses of the blood samples indicated no neutralizing antibodies to OpMNPV. Serum neutralizing antibodies would have been expected if virions had been released from inclusion bodies (intracellular non-occluded virions) in the rat lung. Further, injection of the lung extracts from the rats into the hemocoel of Douglas-fir tussock moth larvae yielded no indication of viral activity, indicating that there was no breakdown of polyhedral inclusion bodies with subsequent viral release in the bronchial and alveolar regions of rat lung.

The effects of intratracheal instillation of TM-Biocontrol was conducted by David (1989c). As in the ocular and dermal studies, this assay used a TM-Biocontrol formulation that was characterized as having a "specified potency of 2.3×10^8 CFU/g". The test material was administered to five Sprague-Dawley rats of each sex at the following doses: 10^6 CFU, 10^5 CFU, 10^4 CFU, and a saline control. Additional groups of five male and five female rats were administered 10^6 CFU of attenuated (i.e., autoclave sterilized) test material. In all cases, the agent was administered in saline at a total volume of 0.04 mL per rat. No treatment related lesions or other signs of toxicity were observed. One male rat in the 10^5 CFU exhibited labored respiration 1 hour after dosing as did two of five females in the 10^6 CFU group.

3.1.8. Subchronic and Chronic Toxicity

The subchronic and chronic toxicity of TM-Biocontrol has not been studied. As noted in the re-registration document (U.S. EPA 1996), the U.S. EPA determined that the very low acute

toxicity of TM-Biocontrol justified waiving the requirement for subchronic and chronic toxicity studies.

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

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

3.1.9. Carcinogenicity and Mutagenicity

No carcinogenicity assays were conducted on TM-Biocontrol. TM-Biocontrol was assayed for its ability to cause chromosomal damage in the Chinese hamster ovary cell system, with and without S-9 activation. At a maximum concentration of 500 mg formulation/L, no effects were noted in the incidence of chromosomal aberrations (Putman and Morris 1989).

3.2. EXPOSURE ASSESSMENT

In the re-registration of both OpNPV and LdNPV, the U.S. EPA (1996) determined that formal exposure assessments for the general public and workers were not required. Two reasons for this decision are given. First, there is essentially no positive hazard identification, and, as subsequently detailed in section 3.3, there is no standard dose-response assessment. In other words, there is no indication that TM-Biocontrol will cause systemic adverse effects; therefore, a formal exposure assessment would serve little purpose. Second,

Spraying of the PIBs of OpNPV and LdNPV will not significantly increase exposure to larval hairs, microbes, or other by-products that occur in the

preparation of the ais [active ingredients]. Pest densities that necessitate spraying have a natural high background of these factors; moreover, dilution of the ais in the spraying preparation and its sticking to the forest foliage reduce the likelihood of exposure to a negligible level. (U.S. EPA 1996, p. 17)

In other words, the use of either TM-Biocontrol or Gypchek will reduce exposure to both the viruses in these products and the insects that they control.

The potential for TM-Biocontrol to reduce exposure to both the OpNPV and the moth larvae can be discussed in some detail. As summarized in section 2.2, the application rate of TM-Biocontrol is 6.8387×10^{12} PIBs/acre. In the production of TM-Biocontrol, the average yield is 6.7×10^8 PIBs per larva (Martignoni 1978). Thus, the number of larval equivalents applied at the nominal application rate is about 10,000 larvae/acre [6.8387×10^{12} PIBs \div 6.7×10^8 = $1.02 \times 10^4 \approx 10,000$ larvae/acre]. This is actually a substantial overestimate because it does not consider the removal of insect parts during the production of TM-Biocontrol. By comparison, the numbers of larvae during a severe infestation in California averaged 50 per 1000 square inches with a range of about 10-80 larvae per 1000 square inches (Mason and Thompson 1971, Table 2, p.7). This estimate corresponds to approximately 300,000 larvae/acre:

$$\begin{aligned} 1000 \text{ in}^2 \div 144 \text{ in}^2/\text{ft}^2 &= 6.94 \text{ ft}^2 \\ 50 \text{ larvae}/6.94 \text{ ft}^2 &= 7.2 \text{ larvae}/\text{ft}^2 \\ 1 \text{ acre} &= 43,560 \text{ ft}^2 \\ 7.2 \text{ larvae}/\text{ft}^2 \times 43,560 \text{ ft}^2 &= 313,632 \text{ larvae.} \end{aligned}$$

Thus, treatment during a severe infestation would increase exposure to the larvae by only about 3% [$10,000 \div 313,632 = 0.032$]. Treatment of an area with a lower infestation rate would reduce exposure by inhibiting the increase in the larval population by a substantial amount with a subsequent reduction in OpNPV exposure. This is consistent with the observations that levels of OpNPV in soil are greater in the untreated areas than in areas treated with OpNPV (Thompson 1975, Thompson and Scott 1979).

3.3. DOSE-RESPONSE ASSESSMENT

There is no basis for conducting a dose-response assessment for systemic toxic effects because no systemic toxic effects can be qualitatively identified for plausible routes of exposure (i.e., oral, dermal, or inhalation). Nonetheless, TM-Biocontrol may cause skin and eye irritation, and these endpoints are of concern at least for occupational exposures. This judgment is consistent with the assessment made by U.S. EPA (1996) in the re-registration of TM-Biocontrol and Gypchek.

Both TM-Biocontrol (David 1989b) and Gypchek (Kuhn 1997a) are moderately irritating when assayed at full strength (TGAI) in the rabbit eye (see section 3.1.6). In the RED, the U.S. EPA (1996) noted the requirement for the following label warning concerning eye irritation for TM-Biocontrol and Gypchek:

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

On review of the study using 2X Gypchek (Kuhn 1997b) in which no eye irritation was noted (see section 3.1.6), the U.S. EPA (Williams 1998) revised this assessment and concluded that:

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

While the Kuhn (1997b) study demonstrated that the 2X dilution of Gypchek is non-irritating, it does not necessarily follow that a 2X or even a 1X (field dilution) solution of TM-Biocontrol will be non-irritating. The above assessment by Williams (1998) assumes that Gypchek and TM-Biocontrol are equally irritating, and this assumption may be evaluated by comparing the TGAI studies on Gypchek (Kuhn 1997a) and TM-Biocontrol (David 1989a) (i.e., the studies in which both of the formulations were tested at full strength). These two studies are reasonably comparable. Both studies involve the application of 0.1 g of the TGAI product to groups of six rabbits without washing the eyes after exposure. Observations were then made at 1 hour post-application and periodically thereafter for 17 days (Kuhn 1997a) to 21 days (David 1989a).

Each study reports results for standard endpoints including clouding of the cornea (corneal opacity), inflammation of the iris (iritis), and swelling of the conjunctiva (conjunctival chemosis). Comparisons of the results for each of these endpoints for both TM-Biocontrol (diamond symbol) and Gypchek (square symbol) are given in Figure 3-1 (conjunctival chemosis), Figure 3-2 (iritis), and Figure 3-3 (corneal opacity).

For all three endpoints, it is apparent that the recovery rate for the eyes of the rabbits exposed to TM-Biocontrol is slower than the recovery rate for the eyes of rabbits exposed to Gypchek. For all three endpoints, the eyes of all of the rabbits exposed to Gypchek were fully recovered over the 17-day observation period (Kuhn 1997a). In the comparable study using TM-

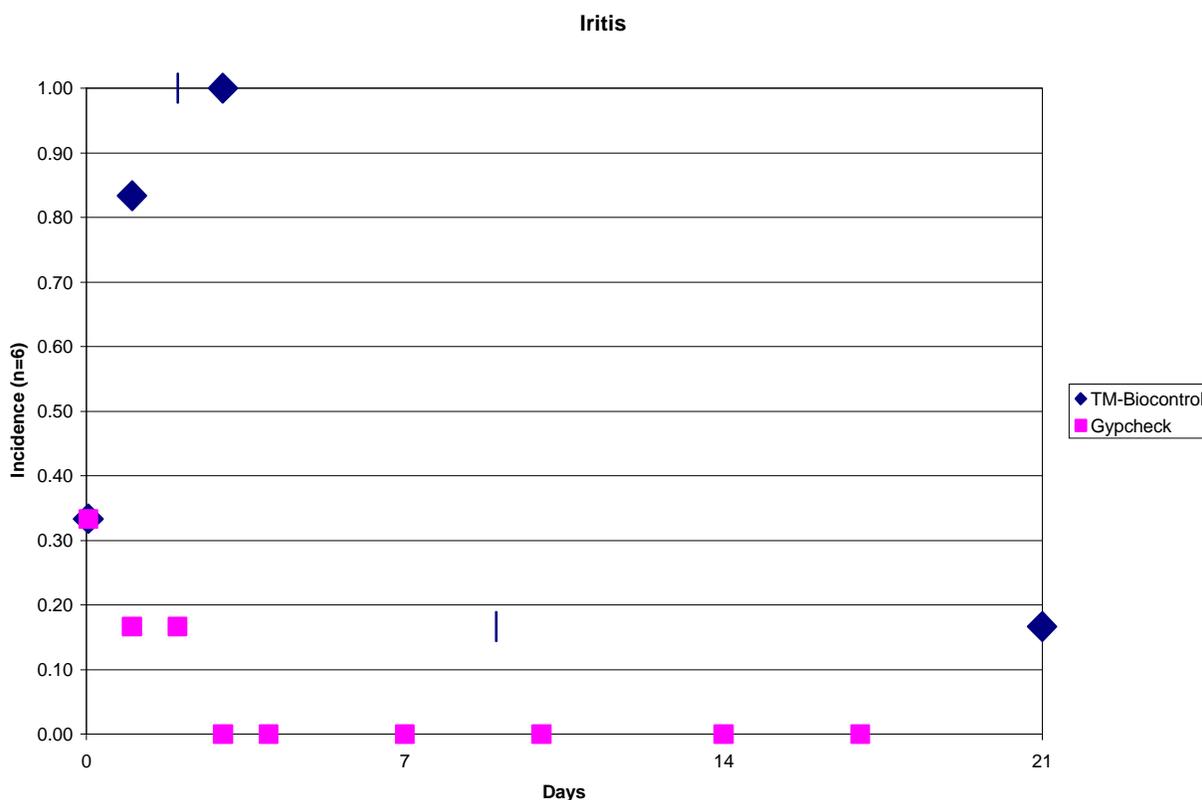


Figure 3-2: Iritis in rabbit eyes after the application of TM-Biocontrol (◆) or Gypchek (■).

Biocontrol (David 1989a), corneal opacity and conjunctival chemosis were present in the majority of the animals at the end of the 21-day observation period. Thus, TM-Biocontrol appears to be more highly irritating to the eyes than Gypchek.

3.4. RISK CHARACTERIZATION

Consistent with the risk characterization presented by the U.S. EPA (1996), there is no basis for asserting that workers are subject to any risk of systemic adverse effects in the use of TM-Biocontrol. Nonetheless, workers involved in the mixing of TM-Biocontrol will be exposed to the undiluted formulation (TGAI) and there is little doubt that there is a potential for skin and eye irritation. The decision by the U.S. EPA (1996) to classify field dilutions of TM-Biocontrol as non-irritating to the eyes is consistent with the decision by the U.S. EPA to “bridge” data between Gypchek and TM-Biocontrol. In that both of these products are NPV and such viruses tend to share many similarities, the decision to bridge data is in many respects reasonable. This approach, however, may be less reasonable for eye irritation. As discussed in section 3.3, there is consistent evidence that TM-Biocontrol has a greater potential than Gypchek to be an eye irritant. Thus, while a 2X solution of Gypchek may be classified as non-irritating, it is less certain that a 2X or even 1X solution of TM-Biocontrol would be non-irritating. Consequently, it would be prudent for workers handling TM-Biocontrol to wear protective goggles to reduce the potential for the introduction of either undiluted formulation

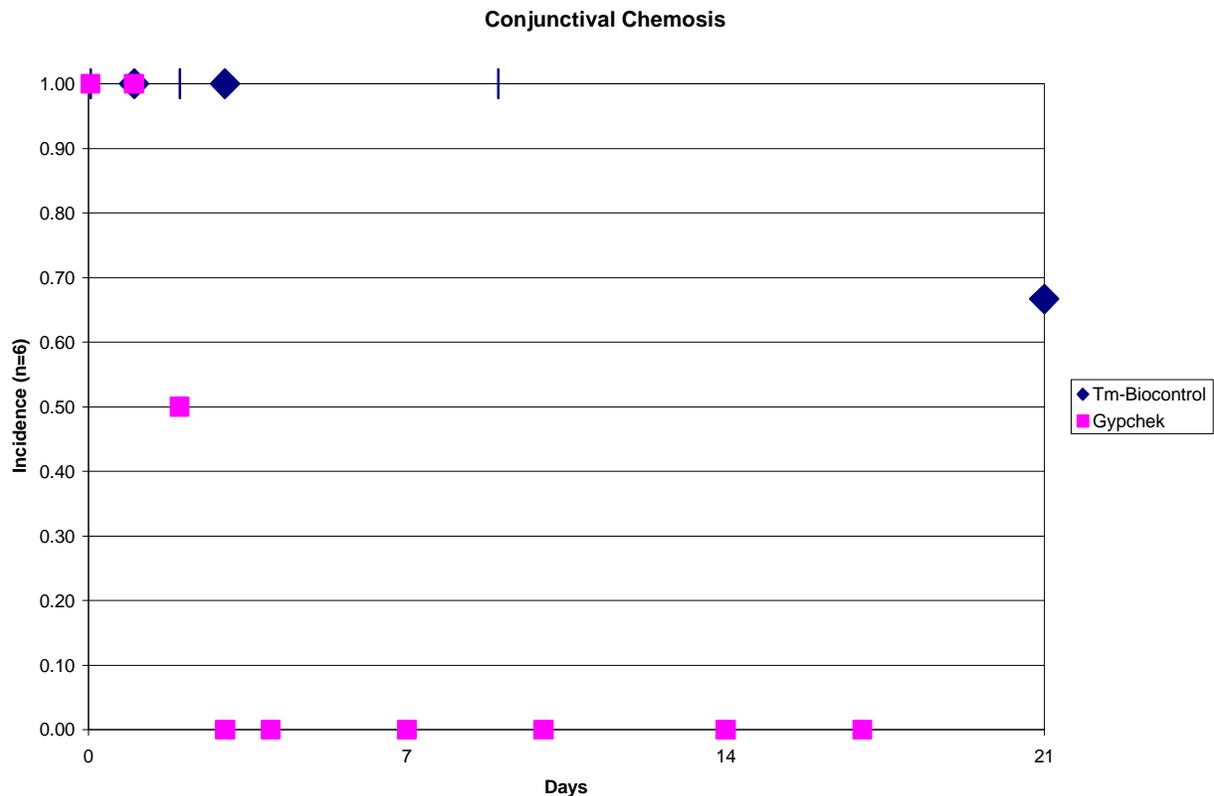


Figure 3-1: Conjunctival chemosis in rabbit eyes after the application of TM-Biocontrol (◆) or Gypchek (■).

or field dilutions of TM-Biocontrol into the eyes.

Workers may also be subject to inhalation of undiluted TM-Biocontrol during mixing but it is less clear if plausible exposures would lead to any adverse effects, based on the inhalation study by Thornett (1975) in which no effects were noted in rats after exposure to concentrations ranging from 0.0049 to 0.79 mg/L of a 70-minute exposure period. Labored respiration was noted in rats after intratracheal instillation of TM-Biocontrol (David 1989c); however, this route of exposure is not particularly likely (i.e., aspiration of undiluted product). Nonetheless, irritation of the respiratory tract as well as labored respiration were reported in studies involving forestry workers exposed to Douglas-fir tussock moth larvae (Perlman et al. 1976), and it is likely that any respiratory effects from exposure to TM-Biocontrol would be attributable to contamination of the OpNPV formulation with larval hairs. Thus, it would seem prudent to caution against mixing TM-Biocontrol in enclosed areas.

Infestations of the Douglas-fir tussock moth tend to occur in relatively remote areas and members of the general public are not likely to be exposed to TM-Biocontrol in the treatment of such infestations. Even if members of the general public were exposed to a spray of TM-Biocontrol, the primary concern would be the insect parts in the formulation. As discussed in

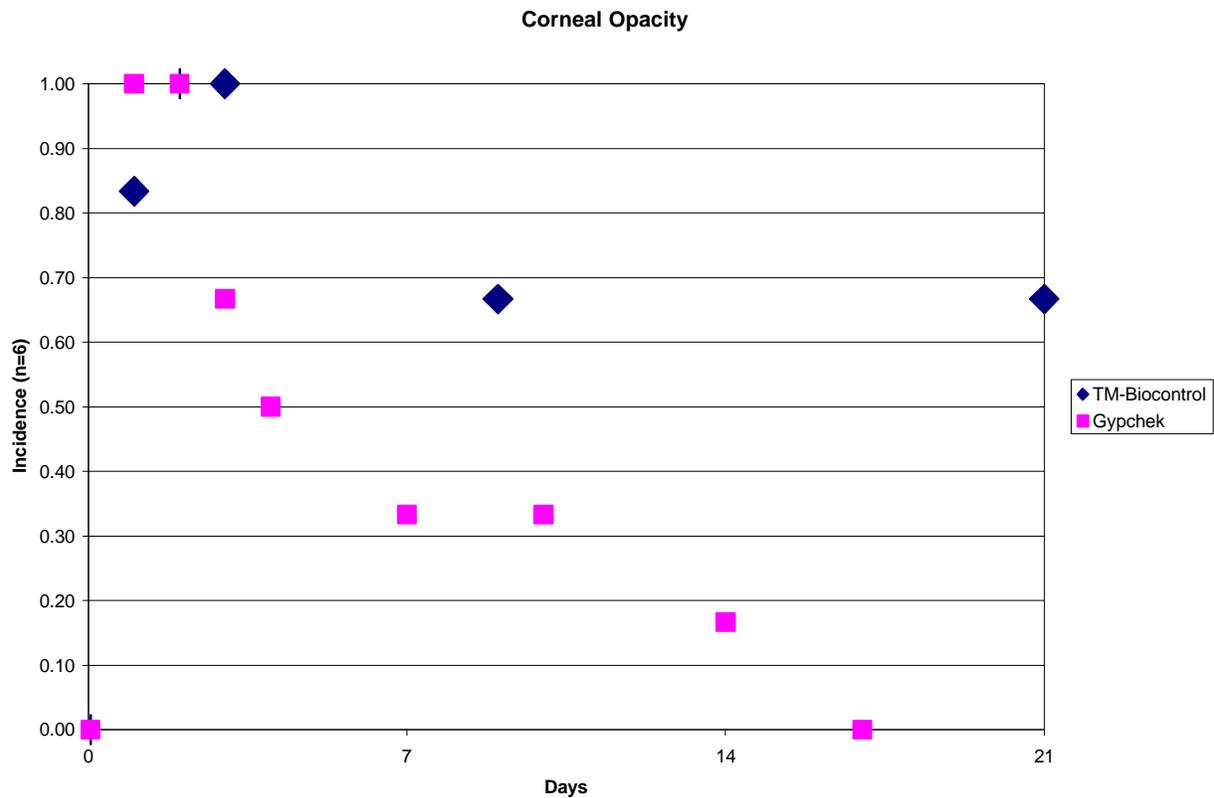


Figure 3-3: Corneal opacity in rabbit eyes after the application of TM-Biocontrol (◆) or Gypchek (■).

section 3.2, applications of TM-Biocontrol for the control of severe Douglas-fir tussock moth infestations will not substantially increase ambient exposure to either OpNPV or the insect parts. To the contrary, the use of TM-Biocontrol will, over the longer term, reduce exposure to OpNPV and the Douglas-fir tussock moth. To the extent that documented health effects of the tussock moth are regarded as a public health issue, the use of TM-Biocontrol could be judged as beneficial.

The available human data (Perlman et al. 1976, Press et al. 1977) suggest that individuals with allergies may be more sensitive to exposures to the Douglas-fir tussock moth than individuals without allergies. For the general public, this issue does not have a substantial impact on the assessment of risk because the use TM-Biocontrol will reduce rather than increase exposure to any allergenic components of the Douglas-fir tussock moth. Potential allergies in workers may be more significant. Workers who are allergic to any components of the Douglas-fir tussock moth would be expected to be more sensitive to TM-Biocontrol than other workers. As with the general public, however, it is likely that exposure to any allergenic components in an area infested with the Douglas-fir tussock moth would be attributable primarily to the moth larvae rather than TM-Biocontrol itself.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Toxicity to Terrestrial Organisms

4.1.1.1. Mammals – One 47.3 kg female mule deer was given an oral dose of 52.9 mg of TM-Biocontrol/kg bw or 1.588×10^9 PIBs/kg bw (Tucker 1966). Other than indicating that a total of 2500 mg of the formulation was administered [$2500 \text{ mg} \div 47.3 \text{ kg} = 52.9 \text{ mg/kg}$], the study does not specify the volume of material administered or the vehicle, if any. On the treatment day, the animal exhibited slight facial reddening and panted more than control animals after running. At one day post-dosing, the animal exhibited yellowish diarrhea which was not evident after 2 days. Moderate neutropenia (decrease in the number of neutral staining white blood cells) was observed in the first few days after treatment and was still evident at 2 weeks post-treatment. As discussed by Tucker (1966), this effect is consistent with systemic viral infection but also may have been due to normal variation. Mild transient eosinophilia (an increase in the number of acid staining white blood cells) consistent with mild foreign protein-histamine reaction was also observed. No PIBs were found in blood smears. One month after exposure, no effects were noted (Tucker 1966).

4.1.1.2. Birds– Tucker (1966) assayed the toxicity of OpNPV after single oral doses to two mallard ducks ($361 \text{ mg/kg} \approx 1.1 \times 10^{10}$ PIBs/kg and $404 \text{ mg/kg} \approx 1.2 \times 10^{10}$ PIBs/kg), one English sparrow ($1969 \text{ mg/kg} \approx 5.9 \times 10^{10}$ PIBs/kg), and one ring-necked pheasant ($384 \text{ mg/kg} \approx 1.2 \times 10^{10}$ PIBs/kg bw). No adverse effects or gross pathological changes were observed in any of the birds over a 2-week observation period (Tucker 1966). Tucker and Crabtree (1970) indicate that the acute oral LD_{50} of an OpNPV formulation, not otherwise specified, was over 3000 mg/kg. They also note, however, the following acute symptoms: *regurgitation*, *dyspnea* [labored respiration], *polydipsia* [excessive thirst], *slight loss of balance*, and *excessive preening*.

In a 5-day oral bioassay, three female mallards were dosed with 50 mg OpNPV/kg bw, equivalent to 9.17×10^8 PIBs/kg bw [$50 \text{ mg OpNPV/kg bw} \times 18.34 \times 10^6 \text{ PIBs/mg}$] in which the test material was administered in gelatin capsules placed into the crop with glass tubing. One of the three females evidenced muscular weakness, characterized only as *myasthenia* in the Tucker (1967) report, within 1 hour of dosing and this symptom persisted throughout the 40-day observation period. A summary of this study in USDA (1988, section 7.1.1 of USDA 1988) indicates that:

Only one of the hens showed muscular weakness within 1 hour after the first treatment, otherwise behaving normally during the 40 day holding period.

The above summary is ambiguous compared to the following statement from the Tucker (1967) study:

Two of the three hens showed no symptoms during the 40-day holding period, while one showed myasthenia within an hour after the first treatment. This symptom persisted throughout the observation period. Section 7.1.3a of USDA 1988

Thus, while the hen may have behaved normally in every respect except muscular weakness, the muscular weakness was apparent throughout the 40-day observation period. In the RED, the U.S. EPA states:

Based on the 90-day response to the DCI [data call in] and additional publicly available literature provided by the USFS, the agency determined that ... data requirements should be waived for 154A-16a Avian oral path/tox--quail, 154A-16b Avian oral path/tox--duck ... (U.S. EPA 1996, p. 6).

The available terrestrial and aquatic data and other relevant scientific information show that the PIBs of LdNPV and OpNPV do not cause adverse pathogenic or toxic effects on avian, mammalian and aquatic wildlife (U.S. EPA 1996, p. 18).

The RED cites the Tucker (1967) study as supplemental but does not discuss the results of or reflect an evaluation of this study.

As summarized in the RED (U.S. EPA 1996), Gypchek, the commercial formulation of LdNPV used to control the Gypsy moth, caused no adverse effects in an 8-day study using mallards exposed to a dietary concentration of 16,000 ppm, no signs of toxicity or pathogenicity to quail after oral doses of 3.73×10^3 PIBs/g/bird, and no signs of toxicity or pathogenicity in chickadees or house sparrows after oral doses of 3×10^7 to 2×10^8 PIBs (U.S. EPA 1996, Table II, p.20).

4.1.1.3. Terrestrial Invertebrates – The Forest Service (USDA 1998) indicates that LdNPV and OpNPV are not known to cause adverse effects in any insects other than the Gypsy moth and the Douglas-fir tussock moth (USDA 1998, Section 885.4340/ 154-23, p. 17). OpNPV does appear to be highly specific to the Douglas-fir tussock moth. Nonetheless, multicapsid OpNPV can replicate in Gypsy moth cells (Bradford et al. 1990). In addition, Sohi et al. (1984) report that OpMNPV can be propagated in and is thus pathogenic to the white-marked tussock moth, *Orgyia leucostigma*. Similarly, Hughes (1976) found that OpNPV is pathogenic to three species in the genus *Orgyia*: the white-marked tussock moth (*O. leucostigma*), the western tussock moth (*O. cana*), and the rusty tussock moth (*O. antiqua*). It is not clear if the SNPVs isolated from *Orgyia leucostigma* (Hayashi 1970, Hayashi and Bird 1970, Sohi et al.

1984) are different from OpSNPV isolated from the Douglas-fir tussock moth. Morris (1964) reported that OpNPV could infect *Lambdina* species but this finding could not be repeated by Hughes (1976).

As indicated in section 2, baculoviruses generally have a very limited number of host species (Chou et al. 1996). Heinz et al. (1995) assayed a recombinant variety of a NPV for *Autographa californica* and found no evidence of adverse effects in two predators of the tobacco budworm, *Chrysoperta carnea* and *Ortus insidiosus*, and no adverse effects on the honey bee (*Apis mellifera*).

Knox (1970) assayed the toxicity of 9 NPVs, including two colonies of OpNPV, to the honey bee, *Apis mellifera*. In this assay, each of the virus strains was mixed with 200 mL of a 1:1 sucrose:water solution. The OpNPV was characterized as 10^{10} PIBs/hive administered over a 4-month period. The report indicates that “no differences were observed between the treated and un-treated colonies” but does not specify the endpoints that were examined or the variability of the endpoints. A summary of this study in USDA (1998) states that:

Knox (1970) conducted a 120 day feeding study and found no effects on egg laying, brood development and honey production when LdNPV and OpNPV were tested at 10,850 activity units/bee. Results were acceptable in Phase 3.

This description is repeated in the RED (U.S. EPA 1996, Table 2, p. 37) and attributed to Knox (1970). The source of the information in the above summary is unclear. Knox is a co-author on a more detailed publication of a series of studies on the effects of various biological insecticides, not including OpNPV, on honey bees (Cantwell et al. 1966). The Cantwell et al. (1966) study did assay for endpoints other than lethality, including brood development, and specifies that hives were allowed to develop to approximately 10,000 workers bees prior to dosing. If the same procedure was used in the Knox (1970) study, the average dose per bee would be 1,000,000 PIBs/bee [10^{10} PIBs/hive \div 10,000 bees/hive]. The Knox (1970) publication does not give any indication of the relationship between PIBs and activity units. Using the relationship of 91 PIBs/activity unit developed in section 2.1, 1,000,000 PIBs/bee would correspond to 10,989 activity units/bee [$1,000,000$ PIBs/bee \div 91 PIBs/activity unit]. Thus, the above summary is a reasonable estimate of activity units per bee from the data in the Knox (1970) publication, and it is probably reasonable to assume that Knox (1970) looked at non-lethal endpoints including reproductive endpoints. Again, however, the information presented in the above summary cannot be attributed directly to Knox (1970).

OpNPV was also assayed in two Trichopteran (caddis fly) species: *Hydropsyche californica* (eight to nine animals per dose) and an unidentified *Linnephilid* species (three animals per dose) at dose rates corresponding to 10^{10} , 2×10^{10} , and 10^{11} PIBs/acre. No mortality occurred in any of the *Linnephilid* species. Sporadic mortality occurred in *Hydropsyche californica*.

The mortality patterns, however, were not dose-related and were attributed to predation or cannibalism (Barr 1976).

4.1.1.4. Terrestrial Plants (Macrophytes)– No phytotoxicity studies on OpNPV were encountered, and the U.S. EPA waived the requirement for such tests (U.S. EPA 1996). As discussed by Cunningham (1982), several field trails using OpNPV were conducted and no indication of phytotoxicity attributable to OpNPV was noted.

4.1.1.5. Terrestrial Microorganisms– Information regarding the effects of TM-Biocontrol on terrestrial microorganisms was not found in the available literature.

4.1.2. Aquatic Organisms.

4.1.2.1. Fish– Martignoni (1968) provides a brief summary of a study to assay the pathogenicity of OpNPV in rainbow trout fry embryonic cells. The summary indicates that no signs of cytotoxicity were apparent and exposure of the cells to OpNPV did not alter the response of the cells to another virus, not otherwise specified, which is known to be pathogenic to fish.

The potential pathogenicity of OpNPV was examined in two salmonid embryonic cell lines, one isolated from chinook salmon and the other from steelhead trout, at a series of concentrations ranging from 1×10^3 to 4×10^7 PIBs per mL (Banowetz and Fryer 1976, Banowetz et al. 1976). No signs of cytotoxicity were reported during the 24-hour exposure period or 7-day post-exposure observation period.

Banowetz et al. (1976) also summarize studies on *in vivo* exposure of chinook salmon, coho salmon, and steelhead trout after exposure of the fish to water contaminated with OpNPV, food contaminated with OpNPV, as well as the direct intraperitoneal injections of the fish with OpNPV preparations. The water exposures are characterized only as “100-surface acre doses” for 200 fish placed in 4 gallons of water that was aerated and stirred at 18°C. After an 18-hour exposure, the fish were maintained for a 30-day observation period. Similarly, the oral exposures are characterized as “100-acre doses adjusted to the surface area of the tanks” with the same holding conditions and observation periods. The injections involved 20 µL which contained 1.67×10^2 LD₅₀ units in terms of toxicity to the tussock moth. No evidence of adverse effects were noted in any of the exposed groups based on mortality or gross examination at sacrifice. In addition, viable virus could not be recovered from the fish after 24 hours. After 8 hours, viable viruses were recovered from the digestive tract after oral administration and from the kidney, spleen, and liver after intraperitoneal injection. No viable viruses were recovered from the kidney, liver, spleen, or digestive tract after water borne exposure. In addition, Banowetz et al. (1976) indicate that the fish would not eat any of the Douglas-fir tussock moth larvae.

Two other viruses, LdNPV and CfNPV, the spruce budworm (*Choristoneura fumiferana*) NPV, had no adverse effect on rainbow trout after the viruses were fed to the trout in standard

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

4.1.2.2. Other Aquatic Species– Information regarding the effects of OpNPV on aquatic invertebrates, plants, or microorganisms were not located in the available literature.

4.2. EXPOSURE ASSESSMENT

As with the human health risk assessment, a formal exposure assessment for TM-Biocontrol is not justified. As discussed in section 3.2, the application of TM-Biocontrol in areas infested by the Douglas-fir tussock moth will not substantially increase exposure to either OpNPV or the larval parts (e.g., hairs) that contaminate OpNPV. To the contrary, treatment of a Douglas-fir tussock moth infestation with TM-Biocontrol is likely to reduce exposure to both the larval parts and the virus.

4.3. DOSE-RESPONSE ASSESSMENT

Also similar to the human health risk assessment, the hazard identification for TM-Biocontrol is essentially negative (i.e., there is little basis for asserting that TM-Biocontrol poses any risk to non-target species). Consequently, a dose-response assessment is not warranted. As summarized by the U.S. EPA (1996):

The available avian and aquatic data and other relevant literature and information show that PIBs of OpNPV and LdNPV do not cause adverse effects on avian, mammalian and aquatic wildlife. No mortalities were seen when these viruses were fed to mallard ducks, house sparrows, bobwhite quail and black-capped chickadees. No mortalities or other adverse effects were seen in brown trout, bluegill sunfish, and a variety of aquatic invertebrates. Similarly, tests with mule deer, Virginia opossums, short-tailed shrews and white-footed mice, resulted in no evidence of pathogenicity or toxicity. Known insect host range and scientific literature on honey bee mortality demonstrate that these baculoviruses do not have adverse effects on honeybees and should not pose a

significant risk to nontarget insects (Cantwell et al. 1972; Knox 1970). NPV effects on endangered species are considered a low risk based on the absence of threat to nontarget organisms. (U.S. EPA 1996, pp. 23-24)

Exposure for some of the species mentioned above involved Gypchek, and the studies on these species are summarized in USDA (1995) and U.S. EPA (1996) but are not summarized in this risk assessment of TM-Biocontrol.

The above summary is not fully consistent with the available information. Apparently, the mentioned *mule deer* refers to the study by Tucker (1966) in which an OpNPV formulation was administered to a single mule deer at a dose of 52.9 mg/kg. As noted in section 4.1.1.1, the observations included gross effects (diarrhea and panting) as well as hematological effects (neutropenia and eosinophilia). Panting may be interpreted as labored respiration, which is consistent with the effects of exposure to TM-Biocontrol in humans and experimental mammals (see section 3). In addition, the investigator judged that the observed hematological changes were consistent with an infection and/or a mild foreign protein-histamine reaction. Thus, this study is not consistent with the assertion of “*no evidence of pathogenicity or toxicity*”.

Similarly, the U.S. EPA cites Tucker (1967) but does not address the effects noted by Tucker (1967) in one of three mallard ducks administered an OpNPV preparation at a dose of 50 mg TGAI/kg bw (i.e., muscular weakness within 1 hour after the first treatment that persisted throughout the 40-day observation period). If this effect were associated with the OpNPV formulation, the rapid onset of the effect would suggest a toxic rather than infectious cause. Alternatively, since the gelatin capsules containing the formulation were “*inserted into the crop via glass tubing*”, the observed effects could have been incidental to damage caused by the glass tubing. The Tucker (1967) study is very brief and there is no way of further assessing the potential significance of the response in the affected animal.

4.4. RISK CHARACTERIZATION

In the re-registration of OpNPV and LdNPV, the U.S. EPA (1996) concludes that:

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

The current re-evaluation of the available information supports this basic conclusion with some minor reservations.

As in the human health risk assessment, there are basically three general agents that could be of concern in TM-Biocontrol: the virus, the insect parts, and incidental bacterial contamination. There is no indication that OpNPV is pathogenic to species other than the Douglas-fir tussock moth and three other closely related tussock moths. To the contrary, experience with this as well as other related NPVs indicate that these viruses have a very narrow host range. As discussed in section 3.1.3, the Forest Service takes reasonable measures to control for possible incidental contamination of TM-Biocontrol by pathogenic bacteria. The formulation undoubtedly contains bacteria that are endogenous to the Douglas-fir tussock moth, and, while somewhat speculative, these bacteria could account for some of the effects observed in animals. It is more likely, however, that most of the observed effects are attributable to the larval parts that are in TM-Biocontrol. As discussed in section 3.1.2, the larvae of the Douglas-fir tussock moth as well as many other larvae of various lepidopteran species contain hairs that are known to cause irritant and perhaps allergic reactions.

As is also true for the human health risk assessment, the overriding consideration in the risk characterization for non-target species is that the use of TM-Biocontrol will decrease rather than increase exposure to the Douglas-fir tussock moth and OpNPV (see section 3.2). Hence, although TM-Biocontrol may have the potential to cause adverse effects, the potential is most clearly related to the larval parts in the formulation, and controlling outbreaks of the Douglas-fir tussock moth will decrease exposure to the larval parts.

Notwithstanding the above assessment, the repeated dose study in mallards by Tucker (1967) suggests a potential for adverse effects that is not consistent with the general expectation that even relatively high doses of OpNPV preparations should be without prolonged adverse effects. The Tucker (1967) study was obviously a preliminary screen for subchronic toxicity. That an effect was seen in one animal within 1 hour and that the effect persisted for 40 days is not consistent with the other information regarding the effects of TM-Biocontrol. The most likely explanation is that the effect noted in the one animal was incidental to damage caused by dosing (i.e., glass tubing inserted into the crop). The best way to clearly and satisfactorily resolve any lingering uncertainty, however, is to repeat the study or conduct a standard subchronic dietary feeding study in mallards.

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6. GLOSSARY

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

Activity Unit -- For TM-Biocontrol, the gross weight of a commercial preparation (in nanograms) causing 50% mortality in the GL-1 strain of the Douglas-fir tussock moth larvae in a standardized bioassay procedure.

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

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

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

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

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

Capsid -- regularly assembled protein subunits that comprise the basic structure of virions.

Carcinogen -- A chemical capable of inducing cancer.

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

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

Conifer -- An order of the Gymnospermae, comprising a wide range of trees, mostly evergreens that bear cones and have needle-shaped or scalelike leaves; timber commercially identified as softwood.

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

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

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

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

Cytoplasmic polyhedrosis -- the formation of crystalline inclusion bodies (polyhedra) in the cytoplasm of mid-gut epithelial cells of insects. Compare to nucleopolyhedrosis.

Dams -- A term used to designate females of some species such as rats.

Degraded -- Broken down or destroyed.

Dermal -- Pertaining to the skin.

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

Entomopathogenic -- bacterial or viral pathogens in insects.

Envelope -- a lipoprotein bi-layer membrane that surrounds viral genetic material (nucleocapsid).

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

Eosinophilia -- Increase in the number of acid staining white blood cells.

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

Epizootic -- a disease that occurs in a large proportion of an animal or plant population at a given time and causes high mortality or morbidity.

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

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

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

Frank effects -- Obvious signs of toxicity.

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

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

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

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

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

Hematological -- Pertaining to the blood.

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

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

Host specific -- Infecting one or only a very small number of species.

Inclusion body -- a intracellular body containing virions or viral antigenic material that is associated with and formed secondarily to a viral infection.

Infectivity -- The ability of a microorganism or virus to survive/persist in another organism.

In vivo -- Occurring in the living organism.

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

Inerts -- Adjuvants or additives in commercial formulations that do not directly affect the target species.

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

Intraperitoneal -- Injection into the abdominal cavity.

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

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

LC₅₀ (lethal concentration₅₀) -- A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

LD₅₀ (lethal dose₅₀) -- The dose of a chemical calculated to cause death in 50% of a defined experimental animal population over a specified observation period. The observation period is typically 14 days.

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

Macrophyte – Terrestrial plant

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

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

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

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

Necropsy – An examination of a dead body that typically includes gross observations of the major organs - i.e., without microscopic examination.

Neutropenia – Decrease in the number of neutral staining white blood cells.

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

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

No-observed-effect level (NOEL) -- The dose of a chemical at which no treatment-related effects were observed.

Nucleopolyhedrosis – a viral disease in insects in which inclusion bodies form in the nuclei of infected cells.

Ocular -- Pertaining to the eye.

Occluded virus – Virus with a inclusion body.

Parenteral – Any form of injection.

Pathogen – A living organism that causes disease; for example, a fungus or bacterium.

Pathogenicity – The ability of a microorganism or virus to reproduce in another organism and cause damage or disease.

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

Polyhedral Inclusion bodies (PIBs) – inclusion bodies that form within the infected cell.

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

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

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

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

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

Sensitization – A condition in which one is or becomes hypersensitive or reactive to an agent through repeated exposure.

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

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

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

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

Terrestrial – Anything that lives on land as opposed to living in an aquatic environment.

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

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

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

Virion – a mature or morphologically complete virus.

Virus – a submicroscopic agent consisting of genetic material, either DNA or RNA, surrounded by a protein coat, or capsid, and, in some viruses, and outer envelope of lipid and carbohydrates. Viruses reproduce exclusively within living cells and most viruses are relatively specific to certain species and types of cells.

7. SUBJECT INDEX

A

active ingredient 3-6, 3-9, 3-10, 6-1
activity unit 2-2, 4-3, 6-1
acute exposure 6-1
adverse-effect level 6-1
AEL 5-1, 6-1, 6-4
aerial application 2-2
allergy 3-3, 3-4, 3-13, 4-7, 5-6
application rate 2-2, 3-2, 3-9
application method 2-2

B

bees 4-3, 4-5, 5-3
bioassay 2-2, 3-5, 4-1, 5-5, 6-1
birds 3-1, 4-1
blood 3-5, 3-7, 4-1, 6-1, 6-2,
6-3, 6-4
body weight 3-4, 3-5, 3-8

C

capsid 2-1, 3-1, 3-2, 4-2, 5-1,
5-3, 5-6, 5-9, 6-1
carcinogenicity 3-8, 5-4, 6-3
carrier 6-1
chronic exposure 6-1
conjunctiva 3-6, 3-10, 3-11
contaminants 2-2, 3-5, 6-2, 6-3
cornea 3-6, 3-10, 3-11

D

dermal 3-3, 3-4, 3-5, 3-7, 3-9,
5-2, 6-2
dermal irritation 3-5

E

envelope 3-1, 5-7, 6-2, 6-6
eosinophilia 4-1, 4-6, 6-2
epidemiology 6-2, 6-5
epizootic 6-2
eye 3-2, 3-3, 3-6, 3-7, 3-9,
3-10, 3-11, 3-12, 5-2, 5-4,
6-4

F

FEL 6-3
fish 3-1, 4-4, 4-5, 5-1, 5-8
foliage 3-9
formulation 1-1, 2-1, 2-2, 3-4, 3-5,
3-6, 3-7, 3-8, 3-10, 3-11,
3-12, 3-13, 4-1, 6-1, 6-3
frank effect 6-3

G

gavage 3-4, 6-3
general public 3-2, 3-3, 3-8, 3-12, 3-13,
6-5
Gypchek 2-1, 3-3, 3-6, 3-8, 3-9,
3-10, 3-11, 4-2, 4-5, 5-4
Gypsy moth 1-1, 2-1, 3-2, 3-3, 3-8,
4-2, 5-4, 5-9

H

hairs 3-2, 3-3, 3-8, 3-12, 4-5, 4-7
hematological 6-3
histopathology 3-8, 6-3
host specific 1-1, 3-1, 6-3

I

impurities 6-2
inclusion body 6-3, 6-5
infectivity 5-4, 6-3
inhalation 3-4, 3-7, 3-9, 3-12, 5-8, 6-5

intraperitoneal 3-4, 3-5, 4-4, 6-3
 invertebrate 3-1, 4-2, 4-5, 5-1, 6-3
 iris 3-10
 irritant 3-3, 3-4, 3-10, 3-11, 4-7, 6-3
 irritant effect 3-4, 6-3
 irritation 3-2, 3-3, 3-5, 3-6, 3-7,
 3-9, 3-10, 3-11, 3-12, 5-2, 5-4

L

larvae 2-1, 2-2, 3-1, 3-3, 3-4, 3-5,
 3-7, 3-9, 3-12, 3-13, 4-4, 6-1
 LD50 4-4, 6-4
 liver 4-4
 LOAEL 6-4

M

mammal 1-2, 3-1, 4-1, 4-5, 4-6
 metameter 1-1, 3-3, 6-4
 microorganism 3-1, 4-4, 4-5, 6-3
 mixture 3-6, 6-3
 mixing 2-2, 3-11, 3-12
 mutagenic 3-8, 6-4
 mutagenicity 3-8, 6-4

N

necropsy 3-4, 3-6, 6-4
 nucleopolyhedrosis 5-1, 5-2, 5-4, 5-5,
 5-6, 6-2
 neutropenia 4-1, 4-6, 6-4
 nontarget 1-1, 4-5, 4-6, 4-7, 5-3,
 5-4, 5-8, 5-9
 nontarget species 1-1, 4-6, 4-7
 NPV 1-1, 1-2, 2-1, 3-1, 3-4,
 3-5, 3-6, 3-7, 3-8, 3-9,
 3-11, 3-12, 3-13, 4-1, 4-3, 5-2

O

ocular 3-3, 3-6, 3-7, 6-4

P

pathogenicity 4-2, 4-4, 4-5, 4-6, 5-4, 6-5
 pH 1-1, 2-1, 2-2, 3-1, 3-3,
 3-4, 3-7, 3-10, 4-1, 4-6,
 5-2, 5-8, 6-1, 6-5
 pine 6-3
 polyhedral inclusion bodies or
 PIBs 1-1, 2-1, 2-2, 3-1, 3-6, 3-7
 3-8, 3-9, 4-1, 4-3, 4-4,
 4-5, 5-8, 5-9, 6-5

R

reproductive 4-3, 6-5
 respiratory 3-2, 3-3, 3-5, 3-12

S

secondary effects 4-7
 sensitive subgroup 6-5
 sensitization 3-5, 5-9, 6-5
 severity 6-4
 skin irritation 3-2, 3-5, 5-9
 skin sensitization 3-5, 5-9
 spray 2-2, 3-5, 3-8, 3-9, 3-12, 5-2
 sprayer 2-2
 systemic toxicity 3-5, 6-5

T

teratology 6-5, 6-6
 terrestrial plants 4-3
 TGAI 3-5, 3-6, 3-7, 3-9, 3-10,
 3-11, 4-6, 5-4
 trout 4-4, 4-5, 5-4
 Tussock moth 1-1, 2-1, 2-2, 3-1, 3-3,
 3-5, 3-7, 3-12, 3-13, 4-2,
 4-4, 4-6, 5-1, 5-8, 5-9, 6-1

V

vehicle	4-1
vertebrate	3-1, 4-2, 4-4, 4-5, 5-1, 6-3, 6-6
virion	3-1, 3-7, 5-6, 6-1, 6-3