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

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GENERAL ACRONYMS, ABBREVIATIONS, AND SYMBOLS

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

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

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

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

CONVERSION OF SCIENTIFIC NOTATION

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

EXECUTIVE SUMMARY

OVERVIEW

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

PROGRAM DESCRIPTION

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

HUMAN HEALTH RISK ASSESSMENT

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

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

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

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

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

Based on simply physical processes associated with the application of any pesticide, it is possible to construct any number of exposure scenarios for Gypchek. The current risk assessment focuses on one extreme exposure scenario involving the accidental spray of a home garden. While Gypchek is not intentionally applied to such vegetation, the inadvertent spray scenario is plausible. Based on this accidental exposure scenario, the estimated dose to an individual is 0.034 mg Gypchek/kg bw, with an upper range of 0.66 mg Gypchek/kg bw.

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

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

Risk Characterization – There is no basis for asserting that any risk is plausible to either workers or members of the general public in the use of Gypchek to control the gypsy moth. This statement follows from the failure to identify any hazard associated with exposures to Gypchek or LdNPV and is essentially identical to the risk characterization given by the U.S. EPA.

As discussed in both the exposure and dose-response assessments, the current risk assessment extends the U.S. EPA risk assessment by proposing a surrogate acute RfD and presenting a very conservative exposure assessment based on the accidental spray of a home garden. This approach is taken simply to facilitate the comparison of risks (or lack of risk) associated with Gypchek to the risks associated with other agents used to control the gypsy moth. Based on a relatively standard dose-response assessment and very conservative exposure assumptions, plausible exposures to Gypchek are below a level of concern by factors of about 50 to over 750. While more typical exposures – i.e., incidental exposure to Gypchek in water or air – are not

provided, they will be substantially less than the range of accidental exposure scenarios used to quantify risk.

ECOLOGICAL RISK ASSESSMENT

Hazard Identification – Similar to the hazard identification for the human health risk assessment, the hazard identification for nontarget wildlife species fails to identify any adverse effects of concern – i.e., there is no indication that LdNPV or the Gypchek formulation of LdNPV has the potential to cause any adverse effects in any nontarget species. The mammalian toxicity data base for LdNPV is reasonably complete and indicates that LdNPV is not pathogenic or otherwise toxic to mammals. One specific study conducted on wildlife mammals that may consume contaminated gypsy moth larvae indicates no adverse effects in mice, shrews, and opossums. Relative to the large number available studies in mammals, few studies are available in birds but the results of these studies are essentially identical to those in mammals indicating that exposures to LdNPV at levels that are substantially higher than those likely to occur in the environment will not be associated with any adverse effects. Based bioassays of LdNPV on the large number of nontarget insect species and supported by the generally high species specificity of related baculoviruses, the hazard identification for LdNPV in nontarget insects is essentially identical to that in birds and mammals. There is no indication that adverse effects will be caused in nontarget insects at any level of exposure. Relatively few studies have been conducted in fish and aquatic invertebrates but these studies are consistent with studies in terrestrial species and indicate that effects on fish or aquatic invertebrates are unlikely. No data are available on the effects of LdNPV on amphibians, aquatic or terrestrial plants or other microorganisms. While this lack of information does, by definition, add uncertainty to this risk assessment, there is no basis for asserting that effects on these or other organisms are plausible.

Exposure Assessment – In ground or aerial applications, it is likely that a large number of species could be exposed to Gypchek/LdNPV. The need for any formal risk assessment is questionable, however, because neither Gypchek nor LdNPV appear to cause systemic adverse effects. Nonetheless, in an attempt to provide some bases for comparing the potential risks of Gypchek to other agents used to control the gypsy moth, two extreme exposure assessments are developed: one for a terrestrial herbivore consuming contaminated vegetation and the other for aquatic organisms in a small pond directly sprayed with Gypchek at the highest application rate. For the terrestrial herbivore, the dose estimates range from 1.1 mg Gypchek /kg bw to 3.2 mg Gypchek /kg bw. For aquatic organisms, concentrations are expressed in units of PIB/liter because this unit is used in the corresponding toxicity studies. For a small pond directly sprayed with Gypchek at the highest application rate, the estimated initial concentration is 2.5×10^{5} PIB/L. A large number of other less extreme exposure assessments could be developed but these would not alter the assessment of risk since these extreme exposure assessments are substantially below any level of concern.

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

Risk Characterization – There is no basis for asserting that the use of Gypchek to control or eradicate gypsy moth populations is likely to cause any adverse effects in any species other than the gypsy moth. While no pesticide is tested in all species under all exposure conditions, the data base on LdNPV and related viruses is reasonably complete and LdNPV has been tested adequately for pathogenicity in a relatively large number of species, particularly terrestrial invertebrates. LdNPV appears to be pathogenic and toxic to the gypsy moth and only to the gypsy moth.

For Gypchek, quantitative expressions of risk are in some respects more difficult because clear NOEC and LOEC values cannot be defined – i.e., if an agent is not shown to cause an effect, the threshold exposure level is not a meaningful concept. Nonetheless, general but very conservative exposure assessments demonstrate that plausible upper ranges of exposures are clearly below any level of concern by a factor of 1000 for terrestrial species and 30,000 for aquatic species.

1. INTRODUCTION

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

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

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

The human health and ecological risk assessments presented in this document are not, and are not intended to be, comprehensive summaries of all of the available information (e.g., efficacy studies) but are focused on the information that most clearly impacts an assessment of risk. Most of the mammalian toxicology studies and some ecotoxicology and environmental fate studies are unpublished reports submitted to the U.S. EPA as part of the registration or re-registration of LpNPV. Full text copies of studies submitted to the U.S. EPA were kindly provided by U.S. EPA/OPP (n=81). These studies were reviewed and are discussed in this document.

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

2. PROGRAM DESCRIPTION

2.1. Overview

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

2.2. Description and Commercial Formulation

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

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

2.3. Application Methods, Rates, and Mixing

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

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

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

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

2.4. Use Statistics

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

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

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

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

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

3. Human Health Risk Assessment

3.1. HAZARD IDENTIFICATION

3.1.1. Overview

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

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

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

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

3.1.2. Epidemiology Studies and Other Human Data

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

3.1.3. Mechanism of Action (Persistence and Pathogenicity)

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

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

3.1.4. Acute Oral Toxicity

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

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

3.1.5. Subchronic or Chronic Systemic Toxic Effects

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

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

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

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

3.1.6. Effects on Nervous System

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

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

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

3.1.7. Effects on Immune System

With LdNPV or any other biological agent that may be pathogenic, the response of or pathological activity in immunocompromised animals – i.e., animals with impaired immune function – is a concern. In addition, some chemical or biological agents may act as immunotoxicants – i.e., chemical agents that disrupt the function of the immune system. Two general types of immunotoxic effects, suppression and enhancement, may be seen and both of these are generally regarded as adverse. Agents that impair immune responses (*immune suppression*) enhance susceptibility to infectious diseases or cancer. Enhancement or *hyperreactivity* can give rise to *allergy* or hypersensitivity, in which the immune system of genetically predisposed individuals inappropriately responds to chemical or biological agents (e.g., plant pollen, cat dander, flour gluten) that pose no threat to other individuals or *autoimmunity*, in which the immune system produces antibodies to self components leading to destruction of the organ or tissue involved (Durkin and Diamond 2002).

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

3.1.8. Effects on Endocrine System

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

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

3.1.9. Reproductive and Teratogenic Effects

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

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

3.1.10. Carcinogenicity and Mutagenicity

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

3.1.11. Irritation (Effects on the Skin and Eyes)

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

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

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

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

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

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

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

3.1.12. Systemic Toxic Effects from Parenteral Exposure

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

3.1.13. Respiratory Effects and Inhalation Exposures

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

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

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

3.1.14. Impurities and Contaminants

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

3.1.15. Inerts and Adjuvants

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

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

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

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview

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

Based on simple physical processes associated with the application of any pesticide, it is possible to construct any number of exposure scenarios for Gypchek. The current risk assessment focuses on one extreme exposure scenario involving the accidental spray of a home garden. While Gypchek is not intentionally applied to such vegetation, the inadvertent spray scenario is plausible. Based on this accidental exposure scenario, the estimated dose to an individual is 0.034 mg Gypchek/kg bw, with an upper range of 0.66 mg Gypchek/kg bw.

3.2.2. LdNPV and Gypsy Moth Parts in Gypchek

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

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

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

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

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

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

3.2.3. Supplemental Extreme Exposures

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

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

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

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview

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

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

3.3.2. Surrogate RfD for Acute Exposures

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

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

3.3.3. Eye Irritation

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

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

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

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

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

3.4. RISK CHARACTERIZATION

3.4.1. Overview

There is no basis for asserting that any risk is plausible to either workers or members of the general public in the use of Gypchek to control the gypsy moth. As discussed in both the exposure and dose-response assessments, the current risk assessment extends the U.S. EPA risk assessment by proposing a surrogate acute RfD and presenting a very conservative exposure assessment based on the accidental spray of a home garden. This approach is taken simply to facilitate the comparison of risks (or lack of risk) associated with Gypchek to the risks associated with other agents used to control the gypsy moth. Based on a relatively standard dose-response assessment and very conservative exposure assumptions, plausible exposures to Gypchek are below a level of concern by factors of about 50 to over 750. While more typical exposures – i.e., incidental exposure to Gypchek in water or air – are not provided, they will be substantially less than the range of doses in the accidental exposure scenarios used to quantify risk.

3.4.2. Pathogenicity and Systemic Toxicity

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

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

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

3.4.3. Extreme Exposure Scenarios

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

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

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview.

Similar to the hazard identification for the human health risk assessment, there is no indication that LdNPV or the Gypchek formulation of LdNPV has the potential to cause any adverse effects in any nontarget species. The mammalian toxicity data base for LdNPV is reasonably complete and indicates that LdNPV is not pathogenic or otherwise toxic to mammals. One specific study conducted on wildlife mammals that may consume contaminated gypsy moth larvae indicates no adverse effects in mice, shrews, and opossums. Relative to the large number of available studies in mammals, few studies are available in birds but the results of these studies are essentially identical to those in mammals indicating that exposures to LdNPV at levels that are substantially higher than those likely to occur in the environment will not be associated with any adverse effects. Based on bioassays of LdNPV on the large number of nontarget insect species and supported by the general high species specificity of related baculoviruses, the hazard identification for LdNPV in nontarget insects is essentially identical to that in birds and mammals. There is no indication that adverse effects will be caused in nontarget insects at any level of exposure. Relatively few studies have been conducted in fish and aquatic invertebrates but these studies are consistent with studies in terrestrial species and indicate that effects on fish or aquatic invertebrates are unlikely. No data are available on the effects of LdNPV on amphibians, aquatic or terrestrial plants or other microorganisms. While this lack of information does, by definition, add uncertainty to this risk assessment, there is no basis for asserting that effects on these or other organisms are plausible.

4.1.2. Toxicity to Terrestrial Organisms.

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

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

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

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

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

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

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

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

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

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

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

4.1.3. Aquatic Organisms.

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

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

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

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

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

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

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

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview

In ground or aerial applications, it is likely that a large number of species could be exposed to Gychek/LdNPV. Because of the apparently very low toxicity of Gypchek and LdNPV, the need for any formal exposure assessment is questionable. Nonetheless, in an attempt to provide some bases for comparing the potential risks of Gypchek to other agents used to control the gypsy moth, two extreme exposure assessments are developed: one for a terrestrial herbivore consuming contaminated vegetation and the other for aquatic organisms in a small pond directly sprayed with Gypchek at the highest application rate. For the terrestrial herbivore, the dose estimates range from 1.1 mg Gypchek /kg bw to 3.2 mg Gypchek /kg bw. For aquatic organisms, concentrations are expressed in units of PIB/liter because this unit is used in the corresponding toxicity studies. For a small pond directly sprayed with Gypchek at the highest application rate, the estimated initial concentration is 2.5×10^5 PIB/L. A large number of other less extreme exposure assessments could be developed but these would not alter the assessment of risk since these extreme exposure assessments are substantially below any level of concern.

4.2.2. LdNPV and Gypsy Moth Parts in Gypchek

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

4.2.3. Supplemental Extreme Exposures

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

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

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

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

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

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

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

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Overview

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

4.3.2. Qualitative Assessment

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

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

4.3.3. Quantitative Assessments

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

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

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

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

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

4.4. RISK CHARACTERIZATION

4.4.1. Overview

There is no basis for asserting that the use of Gypchek to control or eradicate gypsy moth populations is likely to cause any adverse effects in any species other than the gypsy moth. While no pesticide is tested in all species under all exposure conditions, the data base on LdNPV and related viruses is reasonably complete and LdNPV has been tested adequately for pathogenicity in a relatively large number of species, particularly terrestrial invertebrates. LdNPV appear to be pathogenic and toxic to the gypsy moth and only to the gypsy moth.

Because Gypchek does not appear to cause adverse effects, quantitative expressions of risk are in some respects more difficult because clear NOEC and LOEC values cannot be defined – i.e., if an agent is not shown to cause an effect, the threshold exposure level is not a meaningful concept. Nonetheless, general but very conservative exposure assessments demonstrate that plausible upper ranges of exposures are clearly below any level of concern by a factor of 1000 for terrestrial species and 30,000 for aquatic species.

4.4.2. Qualitative Assessment

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

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

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

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

4.4.3. Quantitative Assessments

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

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

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

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

5. REFERENCES

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

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APPENDICES

Appendix 1: Toxicity of LdNPV in Mammals

Appendix 2: Toxicity of LdNPV in Birds

Appendix 3: Toxicity of Gypsy Moth LdNPV in Nontarget Insects

Appendix 4: Toxicity of Gypsy Moth NPV in Aquatic Invertebrates

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

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

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

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

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

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

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

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

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

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

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

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

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

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

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

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

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

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

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

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

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

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

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

Appendix 1: Gypsy Moth NPV Toxicity in Mammals

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

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

Appendix 2: Toxicity of Gypsy Moth LdNPV to Birds

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

Appendix 2: Toxicity of Gypsy Moth LdNPV to Birds

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

Appendix 3: Gypsy Moth NPV Toxicity in Nontarget Terrestrial Insects

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

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

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