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

Draft
Supplemental Environmental
Impact Statement

Summary
Volume I of IV

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a cooperative approach

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Summary

This volume summarizes the draft supplemental environmental impact statement (SEIS) for the gypsy moth. The complete draft SEIS is available on the Web at http://na.fs.fed.us/wv/eis. Print and CD copies are available from the United States Department of Agriculture (USDA) Forest Service, Northeastern Area State and Private Forestry, 180 Canfield Street, Morgantown, WV 26505-3101, phone 304-285-1523.

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Figure 1.   Caterpillar (UGA1929085) USDA Forest Service Archives, www.forestryimages.org; pupa (UGA2652066) USDA Animal and Plant Health Inspection Service, Plant Protection and Quarantine Archives; www.forestryimages.org; moths (UGA2652079) USDA Animal and Plant Health Inspection Service, Plant Protection and Quarantine Archives, www.forestryimages.org; egg mass (UGA3948096) William M. Ciesla, Forest Health Management International, www.forestryimages.org

Figure 2.   USDA Agricultural Research Service, www.ars.usda.gov/is/kids/suburb/story2/microscope.htm

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Figures and Tables

1 Gypsy Moth in the United States.

The gypsy moth is a significant nonnative forest pest in the United States (*Figure 1*). At least 587 million acres (238 million hectares) of trees are susceptible to gypsy moth feeding and defoliation. Also at risk are countless urban and rural forested areas throughout the country where susceptible plants grow naturally or are planted.

Although both European and Asian strains exist, only the European strain is currently present in the United States. The European gypsy moth was brought to the United States and accidentally released in eastern Massachusetts around 1869. Since then, it has continued to spread into uninfested areas naturally and by artificial movement by people. The Asian strain occasionally has been found in the United States, and has been eliminated whenever it has been found. Unlike European female gypsy moths, which cannot fly, the Asian moth poses a greater risk of spread because females can fly and deposit egg masses miles from where they fed as caterpillars.

The gypsy moth continues to be a problem as it spreads: over the last 100 years history shows that gypsy moth outbreaks cause widespread defoliation, tree mortality, environmental and public health risks, and public outcry to control the outbreaks.

2 Proposed Action, Purpose, and Need.

The Forest Service and the Animal and Plant Health Inspection Service (APHIS), as co-lead agencies, propose to supplement the 1996 Record of Decision (ROD) for the 1995 Environmental Impact Statement (EIS): *Gypsy Moth Management in the United States: a cooperative approach*. The Forest Service and APHIS are proposing to add new treatment options, which are described in Section 4 on the Alternatives Considered. New treatments that were not available when the 1995 EIS was written would provide gypsy moth managers with more flexibility in conducting suppression, eradication, and slow-the-spread projects. Making new treatments available is also expected to improve the gypsy moth management program, because each new treatment developed over the last 30 years has proven to be safer, more cost-efficient, easier to use, and often more effective than older treatments. The supplement also provides new information on the gypsy moth and treatments since the 1995 EIS.

Under the 1996 decision, the three strategies of suppression, eradication, and slow the spread established a management program to address the full spectrum of gypsy moth populations found in the United States. The three strategies complement one another, although they differ in objectives and geographic locations:

Suppression reduces damage caused by outbreak populations of gypsy moth caterpillars in the *generally infested area.*

Eradication prevents establishment of the gypsy moth in *uninfested areas* by eliminating isolated infestations that occur as a result of human movement of this pest.

Slow the Spread (STS) slows the rate of spread of the gypsy moth from the generally infested area and prevents infestation of 8 million acres per year in the *transition area,* thus delaying the impacts and costs that occur as the gypsy moth infests new areas.

Gypsy Moth Life Cycle

Caterpillar

8 weeks during
spring and early summer

Young caterpillars are black, developing double rows of red and blue spots as they mature. Insecticide application usually occurs when both the caterpillars and foliage are in the early stages of development.

Pupa

2 weeks during
spring-summer

The female pupa is larger than the male; both are a dark reddish-brown. Caterpillars pupate in protected areas, increasing the chance of accidental movement of pupae by humans.

Adult

Several days
during summer

Male adults are brown or gray. Feather-like antennae detect the pheromone emitted by the female, which is white with small, black markings.

Egg Mass

9 months
summer-spring

Females lay buff-colored egg masses almost anywhere; because the egg life stage is the longest lasting, this stage is most frequently accidentally moved by humans.

Figure 1. The gypsy moth life cycle has four stages.

Treatments approved for use in the strategies are *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*), Gypchek, diflubenzuron, mating disruption (using disparlure), mass trapping (using disparlure alone or with dichlorvos), and the sterile insect technique.

The current overall USDA gypsy moth management program supports an integrated pest management approach that includes planning, detection, evaluation, monitoring, defining acceptable damage, and using appropriate management practices to prevent or control gypsy moth-caused damage and losses in the United States.

3 Programmatic Nature of the Proposed Action.

Like the 1996 ROD, the decision to be made as a result of this SEIS will be programmatic. It will apply to the overall gypsy moth management program of suppression, eradication, and slow-the-spread projects. Specific decisions to undertake any treatment projects will be made following site-specific environmental analyses conducted and documented in accordance with agency implementing procedures for the National Environmental Policy Act. Project proposals will also be analyzed for compliance with Federal laws, such as the Endangered Species Act and National Historic Preservation Act, as well as any applicable State laws.

4 Alternatives Considered.

To learn the concerns of interested and affected people the preparers of the draft SEIS invited public comments for 45 days via a notice in the Federal Register (69 Fed. Reg. 23492, April 29, 2004) soliciting feedback. The result of the public involvement process and internal scoping within the agencies was the identification of three alternatives.

Alternative 1—No Action.

Alternative 1 would maintain the 1996 decision and the current gypsy moth management program; no new treatments would be added to the approved treatments.

Alternative 2—Add Tebufenozide.

Alternative 2 would add the insecticide tebufenozide to currently approved treatments.

Alternative 3—Add Tebufenozide, and Add Other New Treatments Through the Application of the Protocol (Preferred Alternative).

Alternative 3 would add the insecticide tebufenozide and add other treatment(s) that may become available in the future for managing gypsy moths, to currently approved treatments. A new treatment would be available for use upon the agencies' finding that the treatment poses no greater risks to human health and nontarget organisms than are disclosed in this draft SEIS for the currently approved treatments and tebufenozide.

The protocol for making the necessary finding that a treatment is authorized by this Alternative is as follows:

1. Conduct a human health and ecological risk assessment (HHERA). In this risk assessment review all scientific studies available for toxicological and environmental fate information relevant to effects on human health and nontarget organisms. Use this information to estimate risk to human health and nontarget organisms. Include these four elements in the HHERA: (a) hazard evaluation, (b) exposure assessment, (c) dose-response assessment, and (d) risk characterization. The HHERA will do the following:

- Identify potential use patterns, including formulation, application methods, application rate, and anticipated frequency of application.
- Review chemical hazards relevant to the human health risk assessment, including systemic and reproductive effects, skin and eye irritation, dermal absorption, allergic hypersensitivity, carcinogenicity, neurotoxicity, immunotoxicity, and endocrine disruption.
- Estimate exposure of workers applying the chemical.
- Estimate exposure of members of the public.
- Characterize environmental fate and transport, including drift, leaching to groundwater, and runoff to surface streams and ponds.
- Review available ecotoxicity data including hazards to mammals, birds, reptiles, amphibians, fish, and aquatic invertebrates.
- Estimate exposure of terrestrial and aquatic wildlife species.
- Characterize risk to human health and wildlife.

2. Conduct a risk comparison of the human health and ecological risks of a new treatment with the risks identified for the currently authorized treatments and tebufenozide. This risk comparison will evaluate quantitative expressions of risk (such as hazard quotients) and qualitative expressions of risk that put the overall risk characterizations into perspective. Qualitative factors include scope, severity, and intensity of potential effects, as well as temporal relationships such as reversibility and recovery.

3. If the risks posed by a new treatment fall within or below the range of risks posed by the currently approved treatments and tebufenozide,

publish a notice in the Federal Register of the agencies' preliminary findings that the treatment meets the requirements of Alternative 3. The notice must provide a 30-day review and comment period and must advise the public that the HHERA and the risk comparison are available upon request.

4. If consideration of public comment leads to the conclusion that the preliminary finding is correct, publish a notice in the Federal Register that the treatment meets the requirements of Alternative 3 and, therefore, is authorized by that Alternative for use in the USDA gypsy moth management program. The Forest Service and APHIS will make available to anyone, upon request, a copy of the comments received and the agencies' responses.

Like the 1996 Record of Decision, the decision to be made as a result of this draft SEIS will be programmatic. Decisions to use specific treatments in projects, including new treatments authorized under the protocol in Alternative 3, will be made after site-specific environmental analyses are conducted and documented in accordance with agency NEPA implementing procedures.

5 Issues Identified.

Two issues were derived from public involvement for this draft SEIS:

Issue 1—Risk to human health
Issue 2—Risk to nontarget organisms

The effects of each of the treatments on the identified issues are summarized in Section 8.

6 Risk Assessments and Risk Characterization.

Overview.

The consequences of the treatments in each alternative were determined by risk assessment for each treatment as well as for gypsy moth (no treatment) and by a risk comparison among the treatments and gypsy moth.

A risk assessment provides a logical process for evaluating data and analyzing potential effects of the gypsy moth and treatments. Risk assessments take into account the manner in which treatments are used in gypsy moth projects, including how treatment agents are applied, the amount applied, and the types of areas that receive treatment.

Standard steps in the risk assessment process were followed:

- **Hazard identification**--gathers known information from laboratory and field studies on toxicity of the gypsy moth and treatment agents.
- **Exposure assessment**--describes the nature and magnitude of contact with the gypsy moth and with treatment agents as they are used in gypsy moth treatment projects.
- **Dose-response assessment**--determines how much exposure to the gypsy moth and to treatment agents is needed to produce the response (effect) described in the hazard identification.
- **Risk characterization**--combines information from previous steps to describe the plausibility of observing certain effects of the gypsy moth and of treatments.

Each step in a risk assessment is accompanied by uncertainties, caused by limitations either in the available data or in the ability to relate the data to

scenarios of concern. To compensate for uncertainties, risk assessment results tend to be conservative, meaning they are more likely to overestimate risks than to underestimate them.

Human Health and Ecological Risk Assessments (HHERA) were prepared by risk assessment experts (Syracuse Environmental Research Associates, Inc. [SERA]), using the best available data. The HHERAs also underwent independent technical review by other recognized experts in risk assessment methods, toxicology, and other applicable fields (consultants retained by SERA, and toxicologists and program specialists from APHIS and the Forest Service). The HHERAs cover the issues raised in scoping for this SEIS for both human health (human health assessment portion of HHERA) and nontarget organisms (ecological risk assessment portion of HHERA).

Many uncertainties are inherent in conducting and interpreting risk assessments; however, the data available on the agents covered by the risk assessments, modeling, equations and statistics all taken together with the understanding of uncertainties provide adequate information to characterize the relative hazards associated with the agents evaluated. To compensate for missing data and any uncertainties in the data, numerical uncertainty factors are used in the dose-response assessments for potential human health effects, and conservative assumptions are used in both human health and ecological risk assessments. In addition, it is virtually impossible to precisely calculate an exposure value for every situation that may arise. Therefore, models, equations, and statistical techniques were used to quantify both plausible and extreme exposures and to use ranges of toxicity values to reflect ranges of sensitivity. These ranges for exposure and toxicity are then used to numerically characterize risk with hazard quotients that are typically expressed as central estimates with upper and lower bounds.

HHERAs were prepared for each of the treatments in the alternatives and for the gypsy moth itself. The relative risks of the insecticides and treatments were illustrated in a risk comparison evaluation.

Hazard Quotients.

Risks to human health and to nontarget organisms can be estimated numerically using hazard quotients (HQs). HQs can be calculated only for effects on populations of biotic (living) organisms. The HQ is a screening tool commonly used in risk assessments. The HQ is a ratio of the exposure estimate for a particular and defined situation (labeled or prescribed conditions) for a representative population (human or nontarget species), divided by an effect level (dose or concentration level). The HQ takes into account the inherent toxicity of a substance, as well as its ability to produce specific effects on an organism (or population of organisms), and the degree of exposure. *Table 1* provides the HQs for all of the treatments and for the gypsy moth.

As an example, refer to the upper bound of the HQ for *B.t.k.* for nontarget aquatic species--0.5, in *Table 1*. This HQ was derived from an exposure estimate of 0.24 mg/L, which is calculated as the peak concentration of the *B.t.k.* formulation in water after a direct spray. This exposure estimate serves as the numerator for the HQ. The toxicity value of 0.45 mg/L is the NOEC (no observed effect concentration) from a reproduction study in *Daphnia magna,* an aquatic invertebrate. This toxicity value serves as the denominator for the HQ. Thus, the HQ is calculated as follows:

$$HQ = \text{exposure estimate/toxicity value}$$
$$= 0.24 \text{ mg/L} / 0.45 \text{ mg/L}$$
$$= 0.533\ldots \approx 0.5.$$

Note that the HQ in the above example is rounded to one significant place. This is a common practice in presenting HQ values except for those in which the level of concern is marginally exceeded, i.e., an HQ of 1.45 would be rounded to 1.4 but not to 1.0.

In risk management, the HQ must be used in conjunction with other factors and characteristics of a substance, such as the quality and quantity of substantiating evidence (published scientific literature, data, models, and risk assessments done by others such as industry and universities), the severity of potential adverse effects, and the nature of the affected species and populations.

In some cases numerical expressions of risk (HQs) do not adequately convey the potential for hazard. For example, a high HQ for a mild effect, such as skin rash, is probably more acceptable than a much lower HQ for a more serious effect like neurotoxicity. Therefore, the use of HQ as an expression of risk and "acceptability" requires that a qualitative perspective also be injected into the analysis. Ecological risk assessments often involve considerations of many different species of plants and animals, and abiotic factors, and their interrelationships and interactions. Invariably, few data sets are available, and field studies provide only an overview of the complex interrelationships and secondary effects among species. Human health risk assessments and ecological risk assessments cannot offer a guarantee of safety. Both risk assessments offer a way to estimate the adverse effects and the severity of those adverse effects.

7 Effects of the Gypsy Moth.

Risk to Human Health.

Following exposure to gypsy moth caterpillar hairs *(Figure 2)* during gypsy moth outbreaks, children and others who spend time outside may develop rashes or irritation of the eyes or respiratory tract. Some individuals may develop an allergy to the gypsy moth following repeated exposures over 1 or more years.
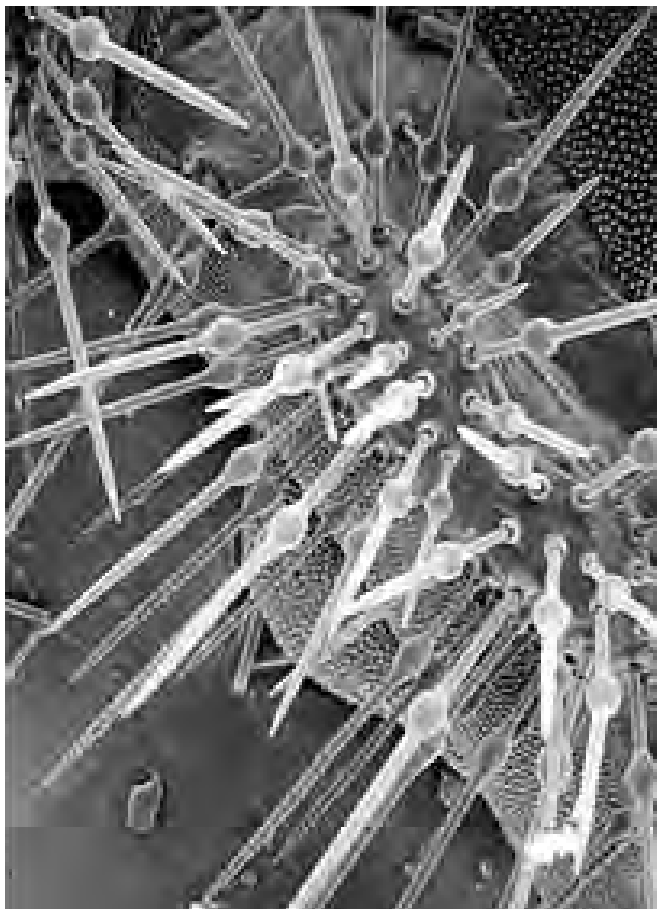
Three years of heavy defoliation cause high mortality in oaks and less-favored hosts; wood growth is drastically reduced, and production of hard mast will likely cease for at least 5 years. Regeneration of young forest to a mature forest requires decades, and in areas where trees less-favored by the gypsy moth remain, stands are dominated by species such as red maple, yellow poplar, black cherry, and yellow or black birch.

Decomposition of leaf fragments and caterpillar droppings reduce oxygen levels in water and result in dramatic increases in algae, reducing acid-neutralizing capacity and increasing watershed yields.

Increased exposure to sunlight caused by defoliation results in seasonal elevations in the temperature of soil and leaf litter, which may temporarily reduce soil moisture content. These factors can lead to short-lived increases in the rates of soil decomposition, mineralization, and plant productivity.

Heavy defoliation can affect animals, fish, and birds. Populations of small mammals (such as the gray squirrel) decline as do some bird species, although woodpecker populations may increase. Trout may decrease in number or disappear from small streams, along with small crayfish and snails. Forest-feeding moths and butterflies, particularly those that feed on oak also are likely to decline, as may other forest-dwelling invertebrates.

8 Effects of Treatments.

All of the treatments described in this SEIS may indirectly help to maintain existing forest conditions and environmental quality by delaying increases in gypsy moth populations, thereby protecting tree foliage.

Figure 2. Gypsy moth hairs can cause irritation.

Risk to Nontarget Organisms.

Environmental effects due to the gypsy moth vary, depending on population levels, the amount of defoliation, and the duration of an outbreak. The most pronounced effects occur when the gypsy moth causes heavy defoliation.. After a single year of heavy defoliation, tree condition suffers and mortality increases. Production of both wood and hard mast (nuts) temporarily declines, and the growth rate of many shrubs and herbaceous plants may increase.

Two years of heavy defoliation greatly reduce the production of wood, hard mast (nuts), and soft mast (berries). Shoestring fungus and two-lined chestnut borer, which attack and kill trees weakened by defoliation, become more abundant. Mortality is likely within 5 years, both among oaks and among species less favored by the gypsy moth.

Currently Approved Treatments.

Bacillus thuringiensis var. *kurstaki* (*B.t.k.*) (Alternatives 1, 2, 3).

Risk to Human Health.

Direct exposure to *B.t.k.* applications may cause some individuals (most likely project workers) to develop minor and transient irritation of the skin, eyes, or respiratory tract. Mating disruption with disparlure will most likely be the only treatment used in the same project area as *B.t.k.* These treatments have different modes of action and are applied weeks apart; therefore, no cumulative effects are expected between *B.t.k.* and disparlure treatments.

Risk to Nontarget Organisms.

Permanent changes in non-target caterpillar populations are not likely following treatment projects that use *B.t.k.* An exception might occur in certain habitat types that support small, isolated populations of moths and butterflies that are highly susceptible to *B.t.k.* Repeated treatment of areas with *B.t.k.* could potentially affect some species of spring-feeding butterfly and moth caterpillars. Since *B.t.k.* is not used in the same locations as other treatments that could affect non-targets, there is no cumulative effect of different treatments with *B.t.k.* on spring-feeding caterpillars

Diflubenzuron (Alternatives 1, 2, 3).

Risk to Human Health.

No human health effects are likely from exposure to diflubenzuron as used in the USDA gypsy moth management program. Exposure to very high levels of diflubenzuron may produce a detectable increase in methemoglobin, an abnormal blood pigment that reduces the oxygen-carrying capacity of blood. Exposure to other methemoglobinemia-inducing compounds in the environment may contribute to a cumulative effect. Individuals exposed to combustion smoke or carbon monoxide (agents that also cause oxidative damage to blood) may be at increased risk of developing methemoglobinemia. Individuals exposed to high levels of nitrates, either in air or in

water, demonstrate increased levels of methemoglobin and may be at increased risk with exposure to compounds such as diflubenzuron. Some individuals have congenital methemoglobinemia and may be at increased risk of adverse effects from compounds that induce methemoglobinemia. Diflubenzuron rapidly dissipates from vegetation and is broken down by sunlight; in the environment the compound degrades to 4-chloroaniline, which the EPA considers a potential carcinogen. This is the only identified potential carcinogen associated with any of the agents to control gypsy moth. The compound is not expected to be present in significant amounts during application since 4-chloroaniline does not form during application. The scenario of greatest concern involving 4-chloroaniline is a cancer risk from drinking contaminated water.

Risk to Nontarget Organisms.

Moth and butterfly caterpillars, grasshoppers, parasitic wasps, some beetles, spiders, sawflies, aquatic insects, and bottom-dwelling and immature free-floating crustaceans may be affected by application of diflubenzuron. Diflubenzuron treatments are applied to the top of the tree canopy and the amount of diflubenzuron residue begins to diminish soon after the application. The population reduction is greater for those species that feed in the upper canopy as compared with those in the mid and lower canopy. Diflubenzuron may cause a reduction in some aquatic invertebrate populations. Diflubenzuron reduces numbers of stream invertebrates that process detritus; however, field studies have shown no decline in detrital decomposition rates. The populations of some invertebrates that feed on algae are reduced by diflubenzuron. An increase in algae could occur after the loss of algal herbivores, however, this has not been observed in field studies.

Birds are not directly affected by exposure to diflubenzuron. Some insectivorous species may show subtle changes, such as a switch in diet, reduced fat

loads, and expanded foraging territories. Similar changes may occur in bats that feed primarily on moths and butterflies.

Diflubenzuron is generally not used in conjunction with other treatments; however, diflubenzuron might be applied to the same area in multiple years for eradication projects. In that case, diflubenzuron might have a cumulative effect on nontarget invertebrates, such as caterpillars of moths and butterflies, grasshoppers, parasitic wasps, aquatic insects, bottom dwelling crustaceans, and immature free-floating crustaceans. Diflubenzuron applications as used in USDA treatment projects will otherwise have no cumulative effects.

Disparlure (as Used in Mating Disruption and Mass Trapping) (Alternatives 1, 2, 3).

Mating disruption entails the aerial application of tiny plastic flakes containing disparlure, the synthetic version of the gypsy moth sex attractant. This treatment confuses male moths and prevents them from locating and mating with females.

Risk to Human Health.

After direct contact with disparlure, a person (most commonly, a project worker) may attract male gypsy moths. Although this attraction could last for years, and could be annoying, there are no data to show it poses any health risk. The general public is not likely to be exposed to sufficient amounts of disparlure to experience this effect. Since disparlure seems to persist in humans, repeated exposures of disparlure will attract the gypsy moth. No information is available on the interaction of disparlure with other control agents or other chemicals usually found in the environment.

Risk to Nontarget Organisms.

Disparlure has low toxicity to vertebrates and is specific to the gypsy moth in North America. As used in mating disruption (and as an attractant in mass trapping), disparlure is not likely to affect populations of non-target organisms. Since disparlure attracts only the gypsy moth in North America, no cumulative effects are expected on nontarget organisms.

Dichlorvos (as Used in Mass Trapping) (Alternatives 1, 2, 3).

Two types of traps are used in mass trapping; both contain disparlure to attract male moths. The smaller delta trap captures moths with a sticky inside surface but contains no dichlorvos. The large milk carton trap contains a pest strip impregnated with the insecticide dichlorvos. Both traps are also commonly used for survey purposes.

Risk to Human Health.

Dichlorvos as used in milk carton traps would pose a health risk to humans only if the individual were to disassemble the trap and tamper with the dichlorvos-impregnated strip. Skin contact with the strip or eating the strip could inhibit the production of acetylcholinesterase. This enzyme prevents the accumulation of acetylcholine, the buildup of which can impair the function of the nervous system. Obvious signs of toxicity to the nervous system are possible but unlikely. Exposure to other substances that inhibit acetylcholinesterase, including similar insecticides, could have a cumulative effect with dichlorvos. The carcinogenic potential of diclorovos has been classified as "suggestive" under the 1999 Environmental Protection Agency Cancer Guidelines.

Risk to Nontarget Organisms.

Invertebrates that inadvertently enter delta or milk carton traps are likely to die. Invertebrates that come into contact with a dichlorvos strip that has accidently fallen on the ground, on vegetation, or in water might also be adversely affected. The potential for adverse effects decreases over time as dichlorvos dissipates from the strip. Large animals, such as bears, that may tamper with traps are not likely to be affected by the dichlorvos strips. Experience with traps used in mass

trapping and survey programs shows that there are no cumulative effects on nontarget organisms even over years of use.

Gypchek (Alternatives 1, 2, 3).

Risk to Human Health.

Irritation of the eyes, skin and respiratory tract is possible from exposure to Gypchek. Gypchek contains gypsy moth parts and may cause irritant effects similar to those caused by the gypsy moth caterpillars. Consequently, exposure to both the gypsy moth caterpillars and Gypchek could be cumulative, although there are no data showing this occurs.

Risk to Nontarget Organisms.

Since Gypchek is specific to the gypsy moth, no effects or cumulative effects on nontarget organisms are expected.

Sterile Insect Technique (Alternatives 1, 2, 3).

The release of sterile insects adds large numbers of sterile gypsy moths to an area, resulting in population reduction and eventual elimination of the infestation.

Risk to Human Health.

The sterile insect technique temporarily increases the number of gypsy moths in the treatment area, increasing both the chance of effects due to the gypsy moth and contact with gypsy moth caterpillars.

Risk to Nontarget Organisms.

No effects or cumulative effects on non-target species are expected.

New Treatment of Tebufenozide (Alternatives 2 and 3).

Risk to Human Health.

Exposure to very high levels of tebufenozide may increase detectable levels of methemoglobin, an abnormal blood pigment that reduces the oxygen-

carrying capacity of the blood. These exposure levels far exceed those exposures expected to occur in project workers and the general public from the USDA gypsy moth management program. The presence of other compounds that raise levels of methemoglobin, such as cigarette or other combustion smoke, carbon monoxide, and nitrates in air or water, may be cumulative. Tebufenozide does not appear to be carcinogenic and does not appear to cause birth defects. No human health effects are likely from exposure to tebufenozide as used in gypsy moth projects.

Tebufenozide and diflubenzuron could have a cumulative effect on methemoglobin but USDA gypsy moth management programs would not use these two chemicals together in the same area at the same time. However, tebufenozide might be applied to the same area in multiple years for eradication projects. These multiple applications of tebufenozide over a period of time may increase the potential risk of methemoglobinemia. Exposure to other methemoglobinemia-inducing compounds in the environment may contribute to a cumulative effect. For example, individuals exposed to combustion smoke or carbon monoxide (agents causing oxidative damage to blood) in addition to exposure to tebufenozide may be at increased risk of developing methemoglobinemia. Individuals exposed to high levels of nitrates, either in air or in water, demonstrate increased levels of methemoglobin and may be at increased risk with exposure to compounds such as tebufenozide.

Risk to Nontarget Organisms.

Tebufenozide may affect other Lepidoptera, especially spring-feeding moths and butterflies. No adverse effects on birds, mammals, or aquatic species are likely to occur from exposure to tebufenozide.

Tebufenozide generally would not be used in conjunction with other treatments. Multiple year applications of tebufenozide might occur for

eradication projects in the same area, but generally these areas are small. Tebufenozide might have a cumulative effect on non-target caterpillars of moths and butterflies, but will not affect other aquatic and terrestrial species.

New Treatments That May Be Available in the Future Under Alternative 3.

Treatments that might become available in the future for managing the gypsy moth cannot be predicted. Given the parameters and protocol built into Alternative 3, any new treatment would pose no greater

risk to human health and nontarget organisms than are disclosed in this SEIS for the currently approved treatments and for tebufenozide.

9 Mitigating Adverse Effects.

Given the variety of places and circumstances where gypsy moth projects could be implemented, it will be necessary to develop and implement specific mitigation measures for each project. Mitigation measures will be developed and implemented on a site-specific basis for each project based on local conditions and concerns.

Table 1. Comparative Hazard Quotients (HQs) for the effects of gypsy moths and treatments on human health and nontarget organisms. Wherever a 0 appears in the table, the hazard quotient value is less than 0.01.

Population	Gypsy Moth HQ	*B.t.k.* HQ	Dichlorvos HQ	Diflubenzuron HQ	Disparlure HQ	Gypchek HQ	Tebufenozide HQ
Human health	1.6 to 625 Upper range is based on major outbreaks	0 to 0.04 Unlikely effects	0 to 380 Upper range based on child tampering with strip.	0.05 to 0.5 – workers, 0.09 to 0.1 – public Upper range for workers based on ground spray operations.	0 No potential risk can be identified	0 to 0.02 No risks are plausible	0.03 to 1.5 Highest HQ based on long-term consumption of contaminated fruit following two applications at the highest application rate.
Nontarget terrestrial species	0.25 to 400 Upper range based on gypsy moth outbreak in sensitive stands	0.36 to 9.4 Upper range based on sensitive caterpillars of moths and butterflies	0 Effects not likely	0.18 to 32 Upper range based on sensitive species of invertebrates	0 No potential hazard identified	0 Effects not likely	0 to 4 Upper range based on the consumption of contaminated vegetation by a large mammal
Nontarget aquatic species	0 No adverse effects	0 to 0.5 Upper level based on sensitive species	0 No risks plausible in normal use. HQ for aquatic invertebrates could reach up to 8 in accidental exposures	0 to 5 Upper range based on acute exposure to aquatic invertebrates (Daphnia)	0 to 0.4 Upper range based on acute exposures to sensitive aquatic invertebrates (Daphnia)	0 No adverse effects	0 to 0.4 Upper range based on longer term toxicity in sensitive aquatic invertebrates

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Gypsy Moth Management in the United States:
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Volume II

Gypsy Moth Management in the United States: a cooperative approach
Draft Supplemental Environmental Impact Statement

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Volume III Appendixes F, G, H, I
Volume IV   Appendixes J, K, L, M

Lead Agency:	Forest Service, U.S. Department of Agriculture (USDA)
Responsible Official:	James R. Hubbard, Deputy Chief for State and Private Forestry Sidney R. Yates Federal Building 201 14th Street, SW Washington, DC 20250
For More Information:	Bill Oldland, SEIS Team Leader 180 Canfield Street Morgantown, WV 26505
Joint Lead Agency:	Animal and Plant Health Inspection Service (APHIS), USDA
Responsible Official:	Richard L. Dunkle, Deputy Administrator for Plant Protection and Quarantine 4700 River Road Riverdale, MD 20737-1236
For More Information:	Weyman P. Fussell, Ph.D., Gypsy Moth Program Coordinator Emergency and Domestic Programs, APHIS 4700 River Road, Unit 134 Riverdale, MD 20737-1236

Abstract: The USDA Forest Service and Animal and Plant Health Inspection Service are proposing an addition to the gypsy moth management program that was described in the 1995 Environmental Impact Statement--Gypsy Moth Management in the United States: a cooperative approach--and chosen in the 1996 Record of Decision. The agencies are proposing these new treatment options: adding the insecticide tebufenozide, or adding the insecticide tebufenozide and other new treatment(s) that may become available in the future to manage gypsy moths, provided that the other treatment(s) poses no greater risk to human health and nontarget organisms than are disclosed in this Draft SEIS for the currently approved treatments and tebufenozide.

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Web Site for Draft SEIS: The Draft SEIS is available for viewing at www.na.fs.fed.us/wv/eis

Send Comments to: Bill Oldland, SEIS Team Leader
                         USDA Forest Service
                         180 Canfield Street
                         Morgantown, WV 26505
                         304-285-1585

Date Comments Must Be Received: See cover letter for the date that comments are due in Morgantown, WV.

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Chapter 1
Purpose of and
Need for Action
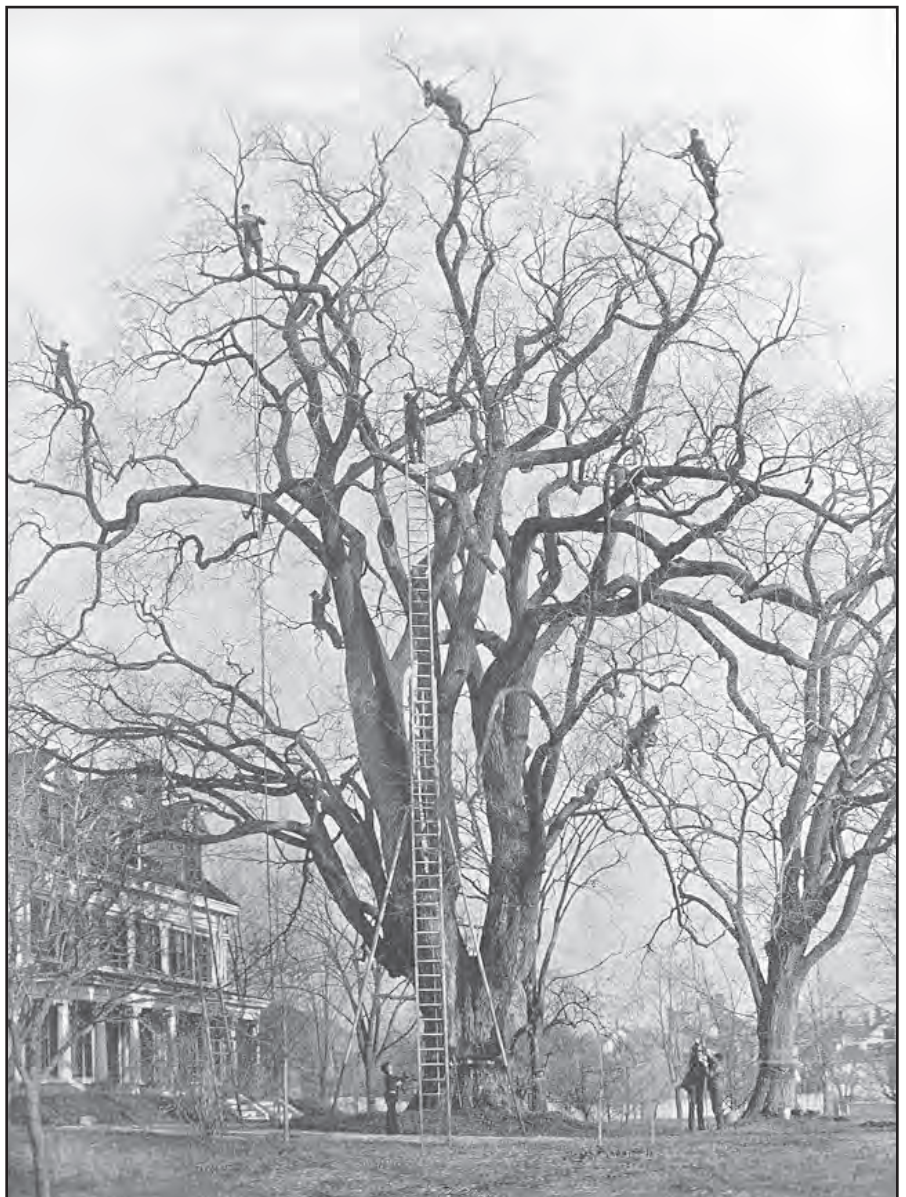
Figure 1-1. In 1892, workers attempted to control gypsy moth by hand picking egg masses.

Chapter 1 Purpose of and Need for Action

Contents

Figures and Tables

The management of gypsy moth in the United States takes an integrated pest management approach to protecting the forests and trees of the United States from the adverse effects caused by the gypsy moth. This chapter gives brief background on the gypsy moth and the current gypsy moth management program. The chapter also states the proposed changes, rationale, and related issues. It explains the purpose of this draft supplemental environmental impact statement (SEIS) and how it is to be used.

1.1 Proposed Action.

The United States Department of Agriculture (USDA) is responsible for management activities related to the gypsy moth (*Lymantria dispar* Linnaeus [L.]), for the Federal government. Two USDA agencies, the Forest Service and the Animal and Plant Health Inspection Service (APHIS) share this responsibility. Agency authorities are found in these USDA Delegations of Authority: 7 Code of Federal Regulations (CFR) 2.60(a)(38) by the Under Secretary for Natural Resources and Environment, for the Forest Service; and <u>7 CFR 2.80(a)(36) by the Under Secretary for Marketing and Regulatory Programs, for APHIS.</u>

The Forest Service and APHIS are proposing an addition to the gypsy moth management program described in the 1995 Environmental Impact Statement (EIS) and chosen in the 1996 Record of Decision (USDA 1995, 1996). The agencies are proposing to add new treatment options: the insecticide tebufenozide and the option of adding other treatments that may become available in the future to manage gypsy moths, provided such treatments pose no greater risks to human health and nontarget organisms than are disclosed in this draft SEIS for currently approved treatments and tebufenozide.

This draft SEIS discloses the method of use, effectiveness, and effects of tebufenozide, and outlines the protocol that would be followed in order to add other treatments. Appendix A provides detailed

information about the use and effectiveness of tebufenozide and other treatments that are effective for eradicating, suppressing, or slowing the spread of the gypsy moth as represented in this draft SEIS. Information about treatments and natural control agents that are not used in the USDA gypsy moth management program is also presented in Appendix A for the benefit of the reader. Appendix B provides an overview of the USDA gypsy moth management program. This draft SEIS also updates effects of currently approved treatments and of the gypsy moth, with new information that has become available since the 1995 EIS, and about the slow-the-spread strategy which is now an operational component of the USDA gypsy moth management program.

1.2 Public Involvement and Issues.

On April 29, 2004, the Forest Service and APHIS published a Notice of Intent (NOI) to Prepare a Supplement to the Final EIS for Gypsy Moth Management in the United States: a Cooperative Approach (69 Federal Register (FR) 23492-93, April 29, 2004). The public was invited to comment on the proposed supplement. Fourteen comment letters were received from the public on the SEIS. Other NOIs were published on March 13, 2006 (71 FR 12674-75) and on February 7, 2007 (72 FR 5675), revising the dates for filing the draft and final SEIS.

The interdisciplinary team preparing this draft SEIS, joined by public affairs specialists and forest pest managers throughout the Forest Service and APHIS (listed in Chapter 5) actively sought public involvement. Two issues were derived from the scoping effort: Issue 1—risk to human health, and Issue 2—risk to nontarget organisms. These issues are described in Chapters 3 and 4 of this SEIS. See Appendix C for details of scoping efforts.

1.3 Background.

The gypsy moth is a significant nonnative forest pest in the United States. The gypsy moth caterpillar—one of four distinct developmental stages (*Figure 1-2*)—alters ecosystems and disrupts people's lives as it feeds on the foliage of trees, shrubs, and other plants. Excessive feeding causes defoliation, which weakens trees (increasing their vulnerability to other insects and diseases that may kill them), alters wildlife habitat, changes water quality, reduces property and aesthetic values of public and private woodlands, and reduces the recreation value of forested areas. When present in large numbers, gypsy moth caterpillars can pose a nuisance, as well as a hazard to health and safety. Effects due to the gypsy moth are described in Chapter 4.

At least 587 million acres (238 million hectares) of trees susceptible to gypsy moth feeding (Appendix D), are at risk in the United States (Powell and others 1993). Also at risk are countless urban and rural forested areas throughout the country where susceptible plants grow naturally or are planted.

Although both European and Asian strains exist, only the European strain is currently present in the United States (*Figure 1-3*). The European gypsy moth was brought to the United States and accidentally released in eastern Massachusetts around 1869. Since then, it has continued to spread into uninfested areas. The Asian strain occasionally has been found in this country, but it has been eliminated whenever it has been found (*Figure 1-4*). Unlike European female gypsy moths, which cannot fly, the Asian moth poses a greater risk of spread because females can fly and deposit egg masses miles from where they fed as caterpillars (*Figure 1-5*).

Despite many early attempts to halt its spread, by 2006 the European gypsy moth became established in the District of Columbia and in all or parts of the following States: Connecticut, Delaware, Illinois, Indiana,

Figure 1-2. Feeding by gypsy moth caterpillars (larvae) causes defoliation.

Figure 1-3. European gypsy moths (male on left, female on right) are found in the United States.

Maine, Maryland, Massachusetts, Michigan, New Hampshire, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Rhode Island, Vermont, Virginia, West Virginia, and Wisconsin (*Figure 1-6)*. Spread continues into uninfested areas because of natural and artificial movement.

The gypsy moth continues to be a problem as it spreads. Historical documentation over the last 100 years reveals gypsy moth outbreaks cause widespread defoliation, tree mortality, environmental and public health risks, and public outcry to control the outbreaks (Williams and Liebhold, 1995a). For more information about the biology, history, and control efforts for the gypsy moth, see Appendix E.

Figure 1-4. This Asian gypsy moth male (left) and female (right) are from Mongolia. As of this writing, the Asian gypsy moth is not found in the United States.

Figure 1-5. People unknowingly spread gypsy moths by moving objects on which egg masses were deposited.

1.4 Purpose of and Need for Action.

In this draft SEIS the Forest Service and APHIS propose to add additional treatments for use in the gypsy moth management program. The proposed treatments are new and were not available when the 1995 EIS was written. Additional treatments would provide gypsy moth managers with more flexibility in conducting suppression, eradication, and slow-the-spread projects. Making new treatments available is also expected to improve the gypsy moth management program, because each new treatment developed over the last 30 years has proven safer to human health and the environment, more cost efficient, easier to apply, and often more effective than older treatments.

This draft SEIS also presents new information about currently used treatments. It…

- Introduces hazard quotients for nontarget organisms
- Reinforces that the gypsy moth poses a significant risk hazard to both human health and forest condition
- Confirms that spring feeding nontarget caterpillars are more at risk from *B.t.k.* applications than are caterpillars that come out later in the year
- Determines that disparlure formulations used for mating disruption are of low toxicity to daphnids
- Makes available additional epidemiological studies for human health effects associated with *B.t.k.*
- Provides data showing that slow the spread is very effective in slowing the natural and artificial spread of the gypsy moth

Figure 1-6. In 2006, the European gypsy moth was established in all or part of 19 states and the District of Columbia (shaded in dark grey).

1.5 Decision Framework.

The 1995 EIS analyzed six alternatives for managing gypsy moth infestations (USDA 1995). With the 1996 Record of Decision (USDA 1996), the agencies selected an integrated pest management (IPM) approach comprised of suppression, eradication, and slow-the-spread strategies to manage the gypsy moth in the United States. The adopted alternative also provides delivery of technical advice and support to State, Tribal, and Federal cooperators by the Forest Service and APHIS. The USDA has carried out its gypsy moth responsibilities under that Record of Decision since 1996.

The 1996 decision provides for the use of several insecticides and other treatments in suppression (*Table 1-1*), eradication (*Table 1-2*), and slow-the-spread projects (*Table 1-3*). These include *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*), the insect growth regulator diflubenzuron, the gypsy moth nucleopolyhedrosis virus product Gypchek, a pheromone attractant disparlure used in mating disruption and mass trapping, the killing agent dichlorvos used in large-capacity pheromone traps, and the sterile insect technique. Human health and ecological risk assessments (HHERA) were prepared for each of these insecticides and for the proposed insecticide tebufenozide, and can be found in Appendixes F-K of this SEIS.

Table 1-1. Acres treated in suppression projects, by treatment, 2002-2006.

Year	*B.t.k.*	Diflubenzuron	Gypchek	Total
2002	149,772	131,601	4,794	286,167
2003	67,895	25,124	10,015	103,034
2004	73,493	0	6,078	79,571
2005	7,292	0	0	7,292
2006	145,053	18,000	602	163,655
Total	443,505	174,725	21,489	639,719

Table 1-2. Acres treated in eradication projects, by treatment, 2002-2006.

Year	*B.t.k.*	Gypchek	Mating Disruption	Total
2002	9,961	0	650	10,611
2003	16,540	0	0	16,540
2004	10,855	0	250	11,105
2005	36,778	0	0	36,778
2006	19,960	0	0	19,960
Total	94,094	0	900	94,994

Table 1-3. Acres treated in slow-the-spread projects, by treatment, 2002-2006.

Year	*B.t.k.*	Gyp-chek	Difluben-zuron	Mating Disruption	Total
2002	28,705	0	3,938	542,600	575,243
2003	70,470	6,819	0	647,618	720,907
2004	131,282	8,230	0	588,256	727,728
2005	108,611	17,075	790	287,890	414,366
2006	95,860	7,003	12,292	426,138	541,293
Total	434,928	39,127	17,020	2,488,502	2,979,577

Like the 1996 Record of Decision, the decision to be made will be programmatic. No site-specific suppression, eradication, or slow-the-spread projects will be implemented as a direct result of the decision that will follow this SEIS. The decision to implement any treatment project will be made after site-specific environmental analyses are conducted and documented in accordance with agency NEPA implementing procedures. Analyses will address unique local issues, beyond the scope of this document, for site-specific management projects for the gypsy moth. Site-specific environmental analyses are more detailed and precise as to geographical locations, individual treatments to be used, and timing of treatments.

The decision on this draft SEIS will serve as the primary guide for management of the gypsy moth on Forest Service lands; treatments and strategies allowed by the 1996 decision will continue to be available for use. The USDA is not reconsidering the suppression, eradication, and slow-the-spread strategies, or the treatments made available by the 1996 Record of Decision. The decision whether to plan and implement a gypsy moth project on National Forest System lands rests with the responsible official in that particular forest.

1.6 Scope of This Document and NEPA Requirements.

This SEIS concerns only the USDA gypsy moth management program carried out by the Forest Service or APHIS, directly or in conjunction with others (States, other Federal agencies, and Tribal governments). Actions of other Federal or local agencies or private citizens to manage the gypsy moth on their own, are not affected or in any way constrained by the USDA program. Such actions are affected or constrained only by applicable Federal and State laws, local ordinances, insecticide label instructions, and any self-imposed constraints.

The information and analysis contained in this SEIS can be incorporated by reference, into environmental documents prepared for proposed gypsy moth management projects, in accordance with the National Environmental Policy Act (NEPA) (42 United States Code (U.S.C.) 4332) and agency NEPA procedures. Future environmental documentation for specific projects would tier to the final SEIS and to the 1995 EIS (40 CFR 1508.28). Proposed treatment projects will be evaluated on an individual basis to determine if they are biologically sound, environmentally acceptable, and economically efficient.

Some gypsy moth related activities, such as treatment of regulated articles infested with gypsy moths, the boarding and inspection of ships entering U.S. seaports, and research and methods-development activities, are outside the scope of this document and were not examined. More information about these activities can be found in Appendix B.

1.7 Consultations.

As they had done on the 1995 EIS, the Forest Service and APHIS will informally consult on the proposed action (Alternative 3) under the Endangered Species Act. In addition, the Forest Service and APHIS will ensure that site-specific consultations will be done as necessary at the project level under the Endangered Species Act, the National Historic Preservation Act, and any other laws, regulations, executive orders, and agency policies that apply to site-specific projects.

Abstract: The USDA Forest Service and Animal and Plant Health Inspection Service are proposing an addition to the gypsy moth management program that was described in the 1995 Environmental Impact Statement--Gypsy Moth Management in the United States: a cooperative approach--and chosen in the 1996 Record of Decision. The agencies are proposing these new treatment options: adding the insecticide tebufenozide, or adding the insecticide tebufenozide and other new treatment(s) that may become available in the future to manage gypsy moths, provided that the other treatment(s) poses no greater risk to human health and nontarget organisms than are disclosed in this Draft SEIS for the currently approved treatments and tebufenozide.

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Chapter 2
Alternatives Including
the Preferred Alternative

Figure 2-1. Early spray operations for gypsy moths used horse-drawn equipment.

Chapter 2 Alternatives Including the Preferred Alternative

Contents

Figures and Tables

This chapter defines the three alternatives that are being considered. It compares the alternatives based on their ability to provide flexibility for managing gypsy moth populations and their relation to the identified issues. The preferred alternative is identified. This chapter also describes mitigation measures that can be used to protect human health and nontarget organisms.

2.1 Background.

The gypsy moth is destructive to vegetative resources, and the human health and environmental effects from exposure to the pest are substantial (Chapter 4 and Appendix L). The strategies of suppression, eradication, and slow the spread and the currently approved treatments (*Table 2-1*) have proven successful in reducing damage caused by gypsy moth outbreaks in the generally infested area, eliminating new isolated infestations of the gypsy moth introduced outside the generally infested area, and slowing the short-range natural and artificial spread of this insect. For a description of the strategies, see Section B-5 in Appendix B.

These strategies form the basis for the alternatives that were considered in the 1995 Environmental Impact statement (EIS) and for the alternatives in this draft supplemental EIS (SEIS).

2.2 Alternative Chosen From the 1995 Gypsy Moth EIS.

A program consisting of the strategies of suppression, eradication, and slow the spread--the preferred alternative in the 1995 EIS--was chosen in the 1996 Record of Decision. The following insecticide and noninsecticide treatments were approved for use in the strategies:

- *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*) (a microbial insecticide)
- Diflubenzuron (an insect growth regulator)
- Gypchek (gypsy moth nucleopolyhedrosis virus product)

- Mass trapping (using traps baited with the gypsy moth attracting pheromone disparlure and sometimes containing the killing agent dichlorvos)
- Mating disruption (aerially dispensed medium impregnated with the gypsy moth attractant disparlure)
- Sterile insect technique (release of sterile or partly sterile gypsy moth pupae or eggs)

Table 2-1 shows which treatments may be used in each strategy.

This alternative was adopted because it fully met the USDA goal of reducing the adverse effects of the gypsy moth on the Nation's forests and trees. The alternative addresses the major issues associated with the gypsy moth and treatments while incorporating flexible options for managing ecosystems affected by the gypsy moth. The issues influencing the discussion in the 1995 Gypsy Moth EIS focused on the effects of the gypsy moth and gypsy moth treatments on human health, nontarget organisms, and forest conditions.

2.3 Alternatives in This SEIS.

Like the 1996 Record of Decision, the decision to be made as a result of this SEIS will be programmatic. No site-specific suppression, eradication, or slow-the-spread projects will be implemented as a direct result of the decision on this SEIS. The decision to implement any treatment project will be made after site-specific environmental analyses are conducted and documented in accordance with agency NEPA implementing procedures.

The following three alternatives were identified during scoping for the draft SEIS:

Alternative 1—No action
Alternative 2—Add tebufenozide
Alternative 3—Add tebufenozide, and add
other new treatments through the application of the protocol **(preferred alternative).**

Table 2-1. Treatments that have been approved for use in gypsy moth projects since the 1995 gypsy moth EIS.

Strategy	B.t.k.	Diflubenzuron	Gypchek	Mass Trapping (Dichlorvos plus disparlure)	Mating Disruption (Disparlure)	Sterile Insect Technique
Suppression	●	●	●			
Eradication	●	●	●	●	●	●
Slow the Spread	●	●	●	●	●	●

Alternative 1—No Action.

Alternative 1 is the same as the alternative selected in the 1996 Record of Decision. It is the current gypsy moth management program of suppression, eradication, and slow the spread, using currently approved treatments. Alternative 1 would make no change to the 1996 Record of Decision, and it would add no treatment options to those approved by that decision.

Alternative 2—Add Tebufenozide.

Alternative 2 would add the insecticide tebufenozide to currently approved treatments. Information on the use and effectiveness of tebufenozide is provided in Appendix A. The human health and ecological risk assessments for tebufenozide are in Appendix J.

Alternative 3—Add Tebufenozide, and Add Other New Treatments Through the Application of the Protocol (Preferred Alternative).

Alternative 3 would add the insecticide tebufenozide and add other treatment(s) that may become available in the future for managing gypsy moths, to currently approved treatments. A new treatment would be available for use upon the agencies' finding that the treatment poses no greater risks to human health and nontarget organisms than are disclosed in this draft SEIS for the currently approved treatments and tebufenozide.

The protocol for making the necessary finding that a treatment is authorized by this Alternative is as follows:

1. Conduct a human health and ecological risk assessment (HHERA). In this risk assessment review all scientific studies available for toxicological and environmental fate information relevant to effects on human health and nontarget organisms. Use this information to estimate risk to human health and nontarget organisms. Include these four elements in the HHERA: (a) hazard evaluation, (b) exposure assessment, (c) dose-response assessment, and (d) risk characterization. The HHERA will do the following:

• Identify potential use patterns, including formulation, application methods, application rate, and anticipated frequency of application.
• Review chemical hazards relevant to the human health risk assessment, including systemic and reproductive effects, skin and eye irritation, dermal absorption, allergic hypersensitivity, carcinogenicity, neurotoxicity, immunotoxicity, and endocrine disruption.
• Estimate exposure of workers applying the chemical.
• Estimate exposure of members of the public.
• Characterize environmental fate and transport, including drift, leaching to groundwater, and runoff to surface streams and ponds.
• Review available ecotoxicity data including

hazards to mammals, birds, reptiles, amphibians, fish, and aquatic invertebrates.

- Estimate exposure of terrestrial and aquatic wildlife species.
- Characterize risk to human health and wildlife.

2. Conduct a risk comparison of the human health and ecological risks of a new treatment with the risks identified for the currently authorized treatments and tebufenozide. This risk comparison will evaluate quantitative expressions of risk (such as hazard quotients) and qualitative expressions of risk that put the overall risk characterizations into perspective. Qualitative factors include scope, severity, and intensity of potential effects, as well as temporal relationships such as reversibility and recovery.

3. If the risks posed by a new treatment fall within or below the range of risks posed by the currently approved treatments and tebufenozide, publish a notice in the Federal Register of the agencies' preliminary findings that the treatment meets the requirements of Alternative 3. The notice must provide a 30-day review and comment period and must advise the public that the HHERA and the risk comparison are available upon request.

4. If consideration of public comment leads to the conclusion that the preliminary finding is correct, publish a notice in the Federal Register that the treatment meets the requirements of Alternative 3 and, therefore, is authorized by that Alternative for use in the USDA gypsy moth management program. The Forest Service and APHIS will make available to anyone, upon request, a copy of the comments received and the agencies' responses.

Like the 1996 Record of Decision, the decision to be made as a result of this draft SEIS will be programmatic. Decisions to use specific treatments in projects, including new treatments authorized under the protocol in Alternative 3, will be made after site-specific environmental analyses are conducted and documented in accordance with agency NEPA implementing procedures.

2.4 Evaluation and Comparison of Alternatives.

Different treatments could be used under the different alternatives, as shown in *Table 2-2*. The more treatments that are available, the more flexibility the program manager has in choosing the right treatment for a given set of specific conditions and the greater likelihood of meeting the project objectives. The Alternatives provide increasing flexibility from Alternative 1 to Alternative 3. With the addition of tebufenozide and other treatments that may become available, Alternative 3—the preferred alternative—would provide the program manager the greatest flexibility. This flexibility for Alternative 3 includes reducing the cost, streamlining the process, and greater efficiency in adding new treatments for gypsy moth management. Cost, availability, efficacy, and site-specific environmental effects are examples of considerations regarding which treatment to use for a specific project.

The effects of the different treatments are summarized by the issues in *Table 2-3*.

Table 2-2. Treatments available under each alternative in this SEIS

Alternative	Currently approved treatments*	Tebufenozide	Other treatments that may become available
1	●		
2	●	●	
3	●	●	●

*Currently approved treatments:
  Bacillus thuringiensis var. *kurstaki*
  Diflubenzuron
  Mass trapping (dichlorvos and disparlure)
  Mating disruption (disparlure)
  Gypchek
  Sterile insect technique

2.5 Mitigation Measures.

Given the variety of places and circumstances where gypsy moth projects could be implemented, it will be necessary to develop and implement specific mitigation measures for each project. Mitigation measures will be developed and implemented on a site-specific basis for each project based on local conditions and concerns.

The site-specific mitigation measures developed and employed in gypsy moth projects since the 1996 Record of Decision have shown to be effective in addressing human health and safety concerns, adverse effects on nontarget organisms and potential impacts on economic resources such as organic farms. At the same time the objectives of gypsy moth projects have been met. Site-specific mitigation measures will continue to be developed and implemented. The following are examples of project level mitigation measures that have been employed in the past and could be implemented for future projects.

Human Health.

● Ensure workers handling insecticides wear appropriate personal protective gear and protective clothing.
● Prepare a project safety plan, disseminate it to project workers, and conduct safety briefings.
● Ensure workers handling dichlorvos insecticide strips wear gloves and assemble the gypsy moth traps outdoors, preferably at the trap site, and transport traps and trapping supplies in an air-tight plastic bag.
● Use gypsy moth traps that do not contain dichlorvos, when possible, in residential areas.
● Encourage public involvement to identify human health issues, including concerns of people sensitive to insecticides. Public notification is an important part of the program, enabling those living in treatment areas to plan their activities and avoid exposure.
● Consider social and cultural factors. Take steps to ensure all groups of the affected population understand the project and are invited to provide input during project development, such as the distribution of information pamphlets in languages relevant to the affected population.
● Give notice to hospitals, schools, public health facilities and local law-enforcement agencies of treatments, the types of insecticides used and risks to humans.
● Give notice of pesticide treatment projects to organizations, groups and agencies that consist of, or work with, people who are chemically sensitive.
● Give notice to the public when treatments are scheduled, including the insecticides planned for use, potential health effects and other characteristics of the project, such as the use of low-flying aircraft.
● Give notice of treatments to people living in the project area sufficiently in advance to allow them to plan their activities and avoid exposure.
● Establish safety and protection measures for workers known to be sensitive to insecticides.
● Establish buffer zones as needed (for example, tebufenozide would not be sprayed over water or areas where surface water is present, and buffers will be maintained around these areas). Certain actions like using the latest advances in application technology as outlined in section A.5 of Appendix A would minimize the risk of insecticides drifting into bodies of water or sites such as organic farms.
● Mix, load, and unload insecticides in areas where an accidental spill will not enter and contaminate bodies of water.

Nontarget Organisms.

● Use public involvement to identify any site-specific issues with potential for effects on nontarget organisms (including threatened and endangered species), and to design appropriate means to mitigate these effects.
● Select treatments taking into consideration maximum project efficiency, potential effects on nontarget organisms (including threatened and endangered species), and the potential for these organisms to recolonize areas if they are displaced or die after treatment.

- Establish buffer zones where necessary to minimize or eliminate insecticide drift to areas of special concern, such as wilderness areas or sensitive species habitats (for example, tebufenozide would not be sprayed over water or areas where surface water is present, and buffers will be maintained around these areas).
- Review maps and conduct ground inspections or other actions as part of the site-specific analysis to identify small brooks, wetlands, estuarine waters, areas where threatened and endangered species are found, bat caves and other roosts or other sensitive areas, and to determine actions needed to minimize adverse outcomes.

- Mix, load, and unload insecticides in areas where an accidental spill will not enter and contaminate bodies of water.

Mitigation Efficacy.

The mitigation measures developed and employed in site-specific gypsy moth projects have proven to be effective in protecting human health and non-target organisms. At the same time, the objectives of gypsy moth suppression, eradication, and slow-the-spread projects have been successfully met since 1996.

Table 2-3. Effects of treatments approved and proposed for use, by alternatives and identified issues. (Unless otherwise noted, the effects are based on the maximum registered usage rate allowed by the insecticide label.)

Treatments and alternatives	Issue 1. Risk to Human Health	Issue 2. Risk to Nontarget Organisms
B.t.k. **Alternatives 1, 2, 3** **See Appendix F for Human Health and Ecological Risk Assessment (HHERA)**	May irritate the eyes, skin, and respiratory tract. Reduces human health effects caused by gypsy moth hairs.	May reduce populations of some spring feeding caterpillars. Reduces effects of gypsy moths on nontarget organisms.
Diflubenzuron **Alternatives 1, 2, 3** **See Appendix I for HHERA**	May slightly increase methemoglobin in sensitive individuals. Reduces human health effects caused by gypsy moth hairs.	Potentially affects arthropod species that produce chitin (hard exoskeleton) and are immature at time of treatment. Can temporarily increase algae due to reduction of algae-feeding aquatic invertebrates. (This has not been observed in the field.) Reduces effects of gypsy moths on nontarget organisms.
Gypchek **Alternatives 1, 2, 3** **See Appendix G for HHERA**	Not likely to affect human health. Reduces human health effects caused by gypsy moth hairs.	Has no effect on nontarget organisms. Reduces effects of gypsy moths on nontarget organisms.
Dichlorvos plus disparlure (Mass Trapping) **Alternatives 1, 2, 3** **See Appendixes H and K for HHERA**	Used in intact traps, not likely to affect human health. Could impair the nervous system if someone disassembles a milk carton trap and tampers with the dichlorvos-impregnated strip, resulting in skin contact or ingestion.	Not likely to affect nontarget organisms.

(continued)

Table 2-3 (continued).

Treatments and alternatives	Issue 1. Risk to Human Health	Issue 2. Risk to Nontarget Organisms
Disparlure (Mating Disruption) Alternatives 1, 2, 3 See Appendix H for HHERA	Not likely to affect human health.	Has no effect on nontarget organisms.
Sterile Insect Technique Alternatives 1, 2, 3	Has no effect on human health	Has no effect on nontarget organisms.
Tebufenozide Alternatives 2, 3 See Appendix J for HHERA	May slightly increase methemoglobin in sensitive individuals. Reduces human health effects caused by gypsy moth hairs.	May affect some Lepidoptera species. Reduces effects of gypsy moths on nontarget organisms.
Other treatment Alternative 3	Has effects no more severe than those described in this SEIS for currently approved treatments and tebufenozide. Reduces human health effects caused by gypsy moth hairs.	Has effects no more severe than those described in this SEIS for currently approved treatments and tebufenozide Reduces effects of gypsy moths on nontarget organisms.

Chapter 3
Affected Environment

Figure 3-1. Undated historical image of workers involved in a gypsy moth management program.

Chapter 3 Affected Environment

Contents

Figures and Tables

This chapter describes the environment that is or could be affected by the gypsy moth and the USDA gypsy moth management program.
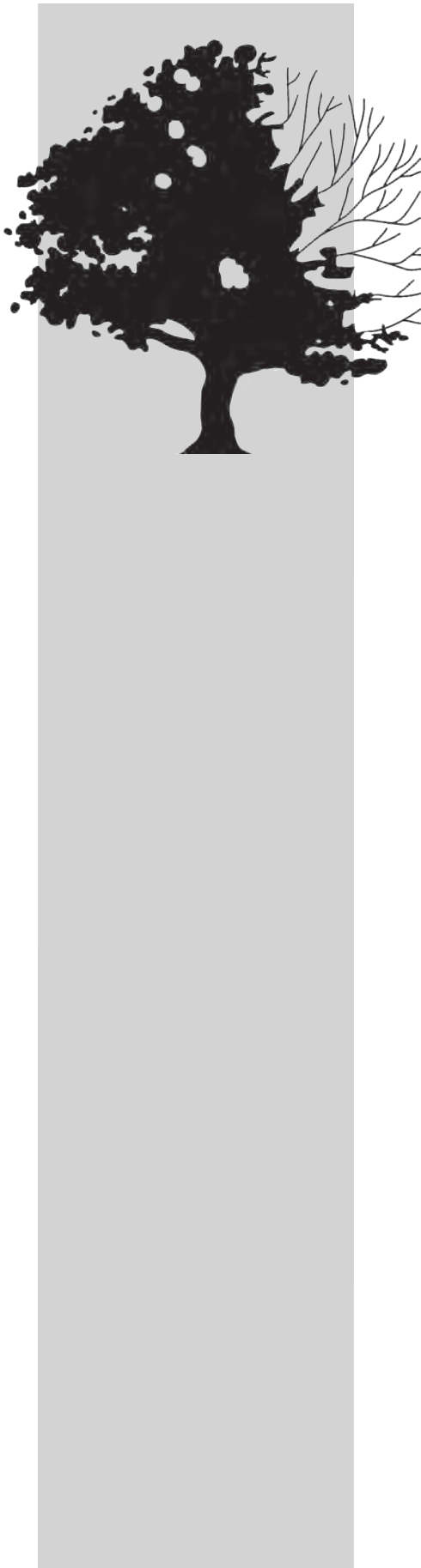
3.1 General Affected Environment.

Because this is a programmatic document, the description of the affected environment contained in this chapter is, by necessity, general. The potentially affected environment in the United States is anywhere vegetation susceptible to gypsy moth feeding is found. Given the known worldwide distribution of the gypsy moth, it is probably capable of surviving anywhere in the United States where suitable host plants are available (McFadden and McManus 1991).

3.2 Affected Forest.

Affected Plants.

Field and laboratory studies of numerous tree species enabled determination of the gypsy moth's feeding preferences (Liebhold and others 1995; and see Appendix D for a list of susceptible plants). Forest trees grow either in pure stands comprised of a single species or in mixed stands as an aggregation of different species. Plant species composition is an important factor in determining the degree of susceptibility of a forest to the gypsy moth (McFadden and McManus 1991). Other factors include total density (basal area per acre) of preferred tree species and proportion of area covered by susceptible stands (*Figure 3-2*). Stands with basal area of preferred species greater than 20 percent are particularly at risk (Liebhold and others 1997).

Table 3-1 lists the total basal area of the 20 most common and important gypsy moth hosts in the United States. The more hardwoods, particularly oaks, in a forest, the more vulnerable it is to the gypsy moth. Higher numbers of susceptible species result in increased intensity, duration, and frequency of defoliation episodes (Davidson and others 1999).

The Forest Service classifies forested areas by combining forest cover types into "forest type groups" for inventory, mapping, and other purposes. Although forest cover types are based on and named after the
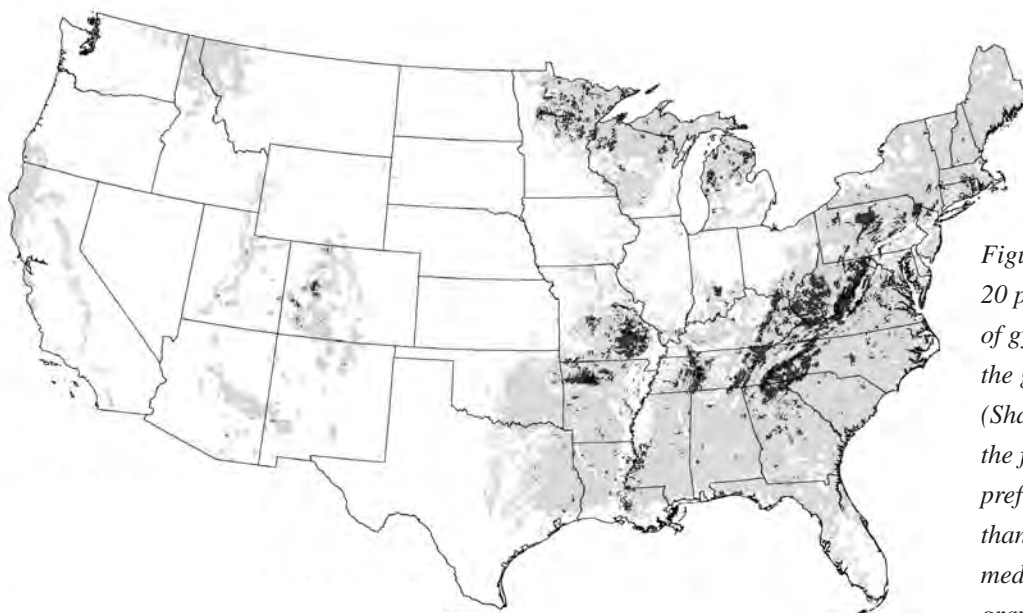
Figure 3-2. Forest stands with 20 percent basal area or more of gypsy moth host trees are at the greatest risk of defoliation. (Shading on the map represents the following basal areas of preferred hosts: white – less than 2%; light gray – 2-20%; medium gray – 21-39%; dark gray – 40-79%)

tree species dominating the stand, other tree species may be present. These associated tree species may be susceptible to the gypsy moth as well.

Oak-hickory is the largest and most diverse susceptible forest type group, extending from the Great Plains to the eastern seaboard. Oak-pine types are found in the South. Oak-gum-cypress types are bottomland forests, typically found in the South and Southeast, especially within the Mississippi Delta and Piedmont. Aspen-birch forests are located in the North Central States. All of these forest types are susceptible to the gypsy moth.

Much of south-central and southeastern Alaska has climate and trees (paper birch, willow and alders) suitable for the gypsy moth. Aspen types are the most abundant hardwood in the intermountain area, while oak types predominate in California and red alder in the Pacific Northwest.

Compared with the European strain, the Asian strain of the gypsy moth feeds on more plants (USDA 1992). In addition to feeding on the same plant species as the European strain, the Asian strain of the gypsy moth will feed on larch and tamarack (*Larix* spp.) in Siberia, eastern Asia, and Japan (USDA 1992), and on both eastern (*L. laricina*) and western larch (*L. occidentalis*) in the United States.

Affected Areas.

Uninhabited Forest.

Land use in uninhabited forest areas is dependent on the individual landowner's management objectives (e.g., timber, wildlife, esthetics, recreation). This classification of forest has no or few residences and few if any paved roads. Uninhabited forest areas exhibit nearly complete forest canopy coverage, typically with three layers composed of subcanopy vegetation, ground layer vegetation, and a layer of organic debris at the

Table 3-1. Top 20 tree species in the United States preferred by gypsy moths, ranked by total basal area (BA).

Common Name	Species	Total BA (100,000,000 ft²)
White oak	*Quercus alba*	14.30
Sweetgum	*Liquidambar styraciflua*	11.60
Quaking aspen	*Populus tremuloides*	10.10
Northern red oak	*Quercus rubra*	9.62
Black oak	*Quercus velutina*	7.31
Chestnut oak	*Quercus prinus*	6.84
Post oak	*Quercus stellata*	5.47
Water oak	*Quercus nigra*	4.34
Paper birch	*Betula papyrifera*	3.81
Southern red oak	*Quercus falcata*	3.75
Scarlet oak	*Quercus coccinea*	3.31
American basswood	*Tilia americana*	2.41
Western larch	*Larix occidentalis*	2.40
Laurel oak	*Quercus laurifolia*	1.94
Bigtooth aspen	*Populus grandidentata*	1.90
Tan oak	*Lithocarpus densiflorus*	1.64
Willow oak	*Quercus phellos*	1.49
California red oak	*Quercus kelloggii*	1.45
Eastern hophornbeam	*Ostrya virginiana*	1.26
Canyon live oak	*Quercus chrysolepis*	1.14

soil level. The layers of vegetation serve to reduce the impact of raindrops and the subsequent chance of erosion due to overland runoff.

Forest Recreation Areas.

Recreation sites typical of rural settings include municipal, county, and state parks, national parks, monuments, forests and grasslands, public and private campgrounds, hiking trails, winter sports complexes, vacation cabins, forest lands for backpacking, and lakes and rivers used for hunting, fishing, and boating. Rural roads and scenic vistas provide attractive and tranquil settings, drawing many visiting tourists from populous, developed areas. All of these areas may be subject to gypsy moth outbreaks.

Forest Residential Areas.

Suppression projects are often conducted in areas where forests and people meet. Examples are forested residential areas that contain single- and multiple-family housing, parks, cemeteries, schools, churches, and small businesses; and woodlots in farm areas that offer the potential for gypsy moth movement. These areas are typically occupied year-round, with landowners directly experiencing the impact of gypsy moth defoliation. Homeowners generally place a high value on their trees for shade, esthetics, privacy, investment, and wildlife habitat, and are consequently concerned when this resource is threatened. Several studies reveal that trees increase property values 5 to 15 percent (Dwyer and others 1992). The presence of defoliated, dying or dead trees can decrease property value and marketability. The cost to remove a dead tree and stump is potentially hundreds of dollars.

Developed Areas.

Natural plant communities in developed areas tend to be fragmented and small, as native plants are frequently replaced with nonnative species.

Forest Condition.

Indicators of forest condition include tree mortality rates, tree growth rates, degree of insect damage (defoliation by gypsy moths), and species composition in the understory and canopy. Gypsy moth defoliation can not only cause mortality of trees, but can also affect the composition of forest communities.

The gypsy moth is not the only introduced pest that can adversely affect the Nation's forest resources. Chestnut blight and Dutch elm disease in the past, and more recently beech bark disease, dogwood anthracnose, emerald ash borer, hemlock woolly adelgid, Asian longhorned beetle, Sirex woodwasp, butternut canker, and others threaten both natural and urban forests. As the gypsy moth and other introduced insects and pathogens spread, they all add stress to forest areas. This stress may be responsible, in part, for documented cases of widespread mortality where no single agent appears to be responsible (Weiss and Rizzo 1987).

Water Quality.

Lakes, streams, rivers and other surface waters in areas with plants susceptible to feeding by gypsy moth caterpillars may be part of the affected environment. Indicators of water quality include flow rate and water chemistry.

Microclimate.

Microclimates created by moisture and temperature conditions found in forests vary by the amount of annual precipitation, elevation, and forest type group. Microclimates may potentially be affected in areas with trees susceptible to gypsy moth feeding.

Soil.

Soil types capable of supporting vegetation susceptible to gypsy moth feeding are potentially part of the affected environment. Soil supports a great diversity of organisms, such as earthworms, arthropods, and microorganisms, which may live in the surface layer, beneath leaf litter, or throughout several soil layers.

Soil structural differences support a wide range of soil-dependent organisms; for example, ground-dwelling arthropods in urban settings are less diverse than those commonly found in undeveloped areas (Gilbert 1989). Impervious surfaces in developed areas prevent air and water from penetrating the soil, which is often more disturbed and compacted than in undeveloped areas. These conditions contribute to a general reduction of plant vigor, root penetration, nitrogen fixation by legumes, and invertebrates to consume and recycle organic matter.

3.3 Affected Human Populations.

Many factors influence the health of people including these: diet, climate, airborne diseases, cultural traditions, emotional well-being, income, access to medical facilities, and contaminants in soil, air, and water. People living in or near areas with trees could be exposed to the gypsy moth and treatments. Particularly susceptible people include those with allergic reactions to gypsy moth hairs (*Figure 3-3*), respiratory ailments, chemical sensitivities, pregnant women, children, and the elderly. Those who work in the woods or with trees, mix or apply insecticides, or work in laboratories with gypsy moths could frequently be exposed to gypsy moths and treatments.

Perceptions and behaviors of individuals vary, depending upon their familiarity with the presence of gypsy moth caterpillars and the use of treatments. Reactions to the gypsy moth are usually strongest where outbreaks occur for the first time; people become alarmed when huge numbers of gypsy moth caterpillars suddenly appear. Perceptions and behaviors in response to the presence of gypsy moth caterpillars and gypsy moth treatment projects may also vary by location. Because urban dwellers are less likely to be exposed to the caterpillars and may never encounter the gypsy moth, they generally do not perceive the moths as being a problem unless the trees in their own yard are directly affected.
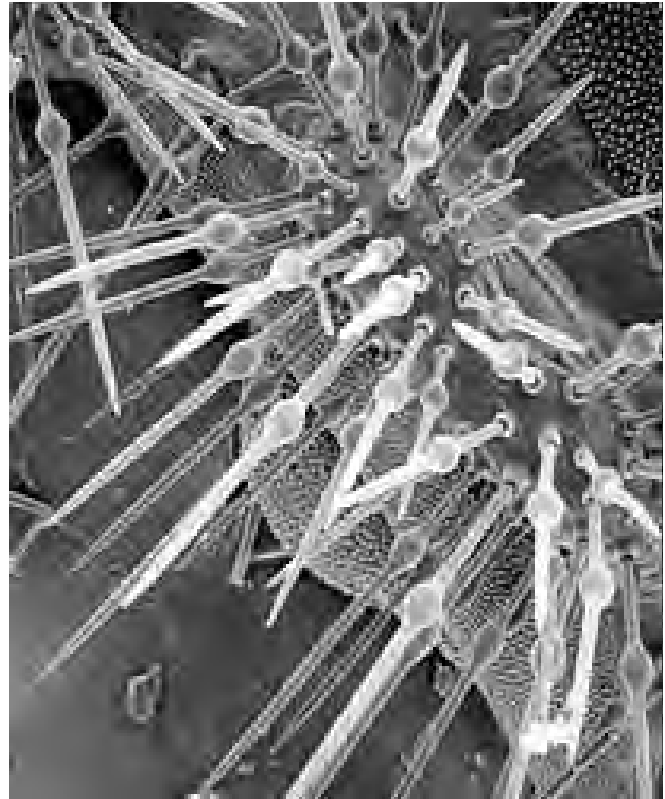
Figure 3-3. Gypsy moth hairs can cause irritation.

Suburban and rural area residents are more likely to be alarmed by large populations of gypsy moth caterpillars and treatment efforts. Inhabitants of rural agricultural areas tend to be less concerned about spraying to control gypsy moth populations due to their familiarity with spraying of agricultural crops.

3.4 Affected Nontarget Organisms.

General.

Virtually all wildlife in the United States that require trees as a part of their environment are within range of the gypsy moth. Mammals, birds, fish, and butterflies, for example, live in environments potentially affected by the gypsy moth or gypsy moth treatments. Detrimental effects of gypsy moths on native Lepidoptera were noted in a West Virginia study (Sample and others 1996).

Animal diversity is generally lower in developed areas, where native animal communities tend to be fragmented and small. Animals that do well in urban or fringe areas usually reproduce rapidly, and exhibit flexible behavior patterns, enabling them to exploit diverse food sources (Gill and Bonnet 1973). Species in urban areas (squirrels and birds like starlings, robins, and crows), which adapt to high human population density, are often found in greater numbers. Domestic animals and pets also comprise a sector of the animal life in areas with high concentrations of people. In contrast, forested areas sustain various populations, including birds (such as warblers, vireos, thrushes, flycatchers, and raptors), as well as large and small mammals such as bobcats and other predators.

Opossum, skunk, raccoon, and squirrel do well in both developed and undeveloped areas, and may be found in areas providing sufficient green space for cover. Larger mammals, such as bear, moose, and wolf, that are sensitive to human disturbances, require larger home ranges and tend to inhabit undeveloped regions.

The diversity of birds is lower in urban settings than in undeveloped areas (Gill and Bonnett 1973). Most bird species in urban areas are year-round residents or short-distance migrants rather than neotropical migrants, which are more common to undeveloped areas.

Reptiles and amphibians do not fare well in developed areas where native vegetation, breeding sites and cover have been disturbed. Loss of habitat, travel barriers and pollution are reasons for fewer numbers of reptiles and amphibians in developed areas than in more natural areas (Campbell 1974a).

Threatened and Endangered Species.

Any species that is listed or proposed for listing as a threatened or endangered species and found in or near forested habitats could potentially be affected by the gypsy moth or gypsy moth treatments. Federally listed species of moths, butterflies, and insect-eating birds are of particular concern.

Chapter 4
Environmental Consequences

Figure 4-1. This experiment station and insectary in Malden, Massachusetts, was used for some of the earliest research on the gypsy moth.

Chapter 4 Environmental Consequences

Contents

Figures and Tables

This chapter examines, on a national scale, the environmental consequences of the alternatives as they relate to the issues of human health and nontarget organisms associated with the treatments that could be used. It updates the general background information presented in the 1995 EIS, and the human health and ecological risk information for the gypsy moth and for currently approved treatments. This chapter also presents human health and ecological risk information for tebufenozide (Alternatives 2 and 3) and other new treatments that may be available in the future (Alternative 3). Any information with a reference date between 1995 and 2006 is new since the 1995 environmental impact statement. All of the information on tebufenozide is new.

4.1 Alternatives and Treatments.

Chapter 2 states the three alternatives. *Table 4-1* lists the treatments that would be available under each alternative.

4.2 Risk Assessments and Risk Characterization.

Overview.

The consequences of the treatments in each alternative were determined by risk assessment for each treatment as well as for gypsy moth (no treatment) and a risk comparison among the treatments and gypsy moth (see Appendixes F-L for the risk assessments, and Appendix M for the risk comparison).

A risk assessment provides a logical process for evaluating data and analyzing potential effects of the gypsy moth and treatments. Risk assessments take into account the manner in which treatments are used in gypsy moth projects, including how treatment agents are applied, the amount applied, and the types of areas that receive treatment.

Standard steps in the risk assessment process were followed:

• Hazard identification—gathers known information from laboratory and field studies on toxicity of the gypsy moth and treatment agents.

Table 4-1. Treatments available for use, by alternative

Treatment	Alternative 1 No action	Alternative 2 Add tebufenozide	Alternative 3 Add tebufenozide and other treatments
*B.t.k.**	●	●	●
Diflubenzuron*	●	●	●
Gypchek*	●	●	●
Mass Trapping (Disparlure, or disparlure and dichlorvos)*	●	●	●
Mating disruption (Disparlure)*	●	●	●
Sterile insect technique*	●	●	●
Tebufenozide		●	●
Other treatments			●

* Currently approved treatments

• Exposure assessment—describes the nature and magnitude of contact with the gypsy moth and with treatment agents as they are used in gypsy moth treatment projects.

• Dose-response assessment—determines how much exposure to the gypsy moth and to treatment agents is needed to produce the response (effect) described in the hazard identification.

• Risk characterization—combines information from previous steps to describe the plausibility of observing certain effects of the gypsy moth and <u>of</u> treatments.

Each step in a risk assessment is accompanied by uncertainties, caused by limitations either in the available data or in the ability to relate the data to scenarios of concern. To compensate for uncertainties, risk assessment results tend to be conservative, meaning they are more likely to overestimate risks than to underestimate them.

Human Health and Ecological Risk Assessments (HHERA) were prepared by risk assessment experts (Syracuse Environmental Research Associates, Inc. [SERA]), using the best available data. The HHERAs also underwent independent technical review by other recognized experts in risk assessment methods, toxicology, and other applicable fields (consultants retained by SERA, and toxicologists and program specialists from APHIS and the Forest Service). The HHERAs and this chapter cover the issues raised in scoping for this SEIS for both human health (human health assessment portion of HHERA) and nontarget organisms (ecological risk assessment portion of HHERA).

Many uncertainties are inherent in conducting and interpreting risk assessments; however, the data available on the agents covered by the risk assessments, modeling, equations and statistics all taken together with the understanding of uncertainties provide

adequate information to characterize the relative hazards associated with the agents evaluated. To compensate for missing data and any uncertainties in the data, numerical uncertainty factors are used in the dose-response assessments for potential human health effects, and conservative assumptions are used in both human health and ecological risk assessments. In addition, it is virtually impossible to precisely calculate an exposure value for every situation that may arise. Therefore, models, equations, and statistical techniques were used to quantify both plausible and extreme exposures and to use ranges of toxicity values to reflect ranges of sensitivity. These ranges for exposure and toxicity are then used to numerically characterize risk with hazard quotients that are typically expressed as central estimates with upper and lower bounds.

HHERAs were prepared for each of the treatments in the alternatives (Appendixes F through K) and for the gypsy moth itself (Appendix L). Results of the HHERAs are summarized later in this chapter. The relative risks of the insecticides and treatments are illustrated in a risk comparison evaluation in Appendix M.

Hazard Quotients.

Risks to human health and to nontarget organisms can be estimated numerically using hazard quotients (HQs). HQs can be calculated only for effects on populations of biotic (living) organisms. The HQ is a screening tool commonly used in risk assessments. The HQ is a ratio of the exposure estimate for a particular and defined situation (labeled or prescribed conditions) for a representative population (human or nontarget species), divided by an effect level (dose or concentration level). The HQ takes into account the inherent toxicity of a substance, as well as its ability to produce specific effects on an organism (or population of organisms), and the degree of exposure. The HQs for currently approved treatments and tebufenozide are described in Appendix M. *Table 4-2* provides the HQs for all of the treatments and for the gypsy moth.

As an example, refer to the upper bound of the HQ for *B.t.k.* for nontarget aquatic species--0.5, in *Table 4-2*. This HQ was derived from an exposure estimate of 0.24 mg/L, which is calculated as the peak concentration of the *B.t.k.* formulation in water after a direct spray. This exposure estimate serves as the numerator for the HQ. The toxicity value of 0.45 mg/L is the NOEC (no observed effect concentration)

Table 4-2. Comparative Hazard Quotients (HQs) for the effects of gypsy moths and treatments on human health and nontarget organisms. (Wherever a 0 appears, the hazard quotient value is less than 0.01.)

Population	Gypsy Moth HQ	*B.t.k.* HQ	Dichlorvos HQ	Diflubenzuron HQ	Disparlure HQ	Gypchek HQ	Tebufenozide HQ
Human health	1.6 to 625	0 to 0.04	0 to 380	0.05 to 0.5– workers 0.09 to 0.1– public	0	0 to 0.02	0.03 to 1.5
(See Table 3-4 of Appendix M for in-depth comments)	Upper range is based on major outbreaks	Unlikely effects	Upper range based on child tampering with strip.	Upper range for workers based on ground spray operations.	No potential risk can be identified	No risks are plausible	Highest HQ based on long-term consumption of contaminated fruit following two applications at the highest application rate.
Nontarget terrestrial species	0.25 to 400	0.36 to 9.4	0	0.18 to 32	0	0	0 to 4
(See Table 4-4 of Appendix M for in-depth comments)	Upper range based on gypsy moth outbreak in sensitive stands	Upper range based on sensitive caterpillars of moths and butterflies	Effects not likely	Upper range based on sensitive species of invertebrates	No potential hazard identified	Effects not likely	Upper range based on the consumption of contaminated vegetation by a large mammal
Nontarget aquatic species	0	0 to 0.5	0	0 to 5	0 to 0.4	0	0 to 0.4
(See Table 4-5 of Appendix M for in-depth comments)	No adverse effects	Upper level based on sensitive species	No risks plausible in normal use. HQ for aquatic invertebrates could reach up to 8 in accidental exposures	Upper range based on acute exposure to aquatic invertebrates (Daphnia)	Upper range based on acute exposures to sensitive aquatic invertebrates (Daphnia)	No adverse effects	Upper range based on longer term toxicity in sensitive aquatic invertebrates

from a reproduction study in *Daphnia magna,* an aquatic invertebrate. This toxicity value serves as the denominator for the HQ. Thus, the HQ is calculated as follows:

HQ = exposure estimate/toxicity value
     = 0.24 mg/L / 0.45 mg/L
     = 0.533… ≈ 0.5

Note that the HQ in the above example is rounded to one significant place. This is a common practice in presenting HQ values except for those in which the level of concern is marginally exceeded, i.e., an HQ of 1.45 would be rounded to 1.4 but not to 1.0.

In risk management, the HQ must be used in conjunction with other factors and characteristics of a substance, such as the quality and quantity of substantiating evidence (published scientific literature, data, models, and risk assessments done by others such as industry and universities), the severity of potential adverse effects, and the nature of the affected species and populations.

In some cases numerical expressions of risk (HQs) do not adequately convey the potential for hazard. For example, a high HQ for a mild effect, such as skin rash, is probably more acceptable than a much lower HQ for a more serious effect like neurotoxicity. Therefore, the use of HQ as an expression of risk and "acceptability" requires that a qualitative perspective also be injected into the analysis. Ecological risk assessments often involve considerations of many different species of plants and animals, and abiotic factors, and their interrelationships and interactions. Invariably, few data sets are available, and field studies provide only an overview of the complex interrelationships and secondary effects among species. Human health risk assessments and ecological risk assessments cannot offer a guarantee of safety. Both risk assessments offer a way to estimate the adverse effects and their severity.

4.3 Consequences of the Gypsy Moth.

This section provides exsisting and updated information on the gypsy moth. It is intended for use with site-specific project analysis and for general information for the reader. See Appendix E for information on the history and biology of the gypsy moth. See Appendixes L and M for detailed analysis of risks associated with gypsy moths.

General Effects of the Gypsy Moth.

Forest Condition–Effects of Defoliation on Vegetation.

When gypsy moth populations are low, nearly all feeding and defoliation occurs on favored hosts, such as oaks (Campbell and Sloan 1977a). During population outbreaks gypsy moth caterpillars feed on more than 300 species of broad-leaved and coniferous trees and shrubs (Leonard 1981) (see Appendix D, Plant List). Trees stripped of 50 percent or more of their leaves are likely to refoliate the same season, although new leaves are fewer and smaller than the originals (Wargo 1981a). The impact of defoliation depends on five key factors:

(1) How much foliage is removed;
(2) The number of successive years of defoliation;
(3) When defoliation occurs in the growing season;
(4) The presence and number of secondary organisms; and
(5) The physiological condition of the tree (Parker 1981).

Defoliated trees already under stress from drought or other factors often succumb more quickly than healthier trees.

After gypsy moth outbreaks red maple (*Acer rubrum*) numbers may increase and oak numbers decrease in Appalachian forests (Allen and Bowersox 1989, Gansner and others 1994, Hix and others 1991), because red maple is not a preferred host and oaks are preferred. Trends in New England and Pennsylvania reveal a shift in composition towards less oak, with

some stands having major losses and others having only minor changes (USDA Forest Service 1994f). Moderate-to-heavy defoliation accelerates forest succession towards more shade-tolerant (and less defoliation-prone) species (Campbell and Sloan 1977a, Clement and Nisbet 1972, Feicht and others 1993, Houston 1981b, Stephens and Hill 1971).

An area that is defoliated for only 1 year will have minimal long-term effects. However, defoliation by even non-epidemic levels of gypsy moth larvae could have a significant, negative effect on the radial growth of preferred trees, except possibly aspen (Muzika and Liebhold 1999, Naidoo and Lechowicz 2001). Small feeder roots die, reducing water and mineral uptake and slowing tree recovery (Wargo 1978b). The effects of a single heavy defoliation in a mixed stand of oaks in eastern New England were visible for 10 years (Campbell and Sloan 1977a). Decreases in stem volume growth in southern New England averaged approximately 20 percent in any year a tree was defoliated compared with no defoliation the previous year, and growth loss was evident up to 3 years after defoliation (Twery 1987, Wargo 1981a). Overall stand volume may decrease initially (Gansner and Herrick 1982, Herrick and Gansner 1988) and then may increase over time (Gansner and others 1993b).

Defoliation reduces carbohydrate (starch) production (Heichel and Turner 1976, Kozlowski 1969) forcing trees to use root starch reserves. Most trees can tolerate 2 years of defoliation before root starch reserves are depleted (Wargo 1981a). Depletion of reserves weakens trees, making them vulnerable to secondary organisms that cause further decline and death. In the eastern United States the principal secondary organisms are the shoestring fungus (*Armillaria mellea*) and the two-lined chestnut borer (*Agrilus bilineatus)* (Houston 1981a, Wargo 1981b).

Increased light due to defoliation causes herbaceous plants to rapidly expand their density and coverage (Gottschalk 1988). In some areas that are subject to intense deer browsing, defoliated trees may fail to regenerate, and shrubs or herbaceous plants can dominate (Gottschalk 1988).

Heavy defoliation by the gypsy moth increases fire danger (Gottschalk 1990a). An abundance of heavy fuel, standing dead snags, dense understory vegetation, and numerous fallen trees act in combination to promote spot fires, impede fire line construction, and extend the time needed for post-fire mop-up operations (Tigner 1992).

Forest Condition—Tree Mortality.
Several factors interact to produce tree and stand mortality: severity, frequency, and distribution of defoliation, site and stand factors, environmental conditions, tree vigor, crown condition, and presence and abundance of secondary organisms (Campbell and Valentine 1971, Kulman 1971, Staley 1965, Campbell and Sloan 1977a, Gansner and others 1978, Wargo 1978a, b, Campbell 1979, Herrick and Gansner 1987, Fosbroke and Hicks 1989, Herrick 1982, Tigner 1992, Feicht and others 1993, Gottschalk and MacFarlane 1993). Oak mortality in initial outbreaks is greater than in later outbreaks (Davidson and others 1999). Oaks and other susceptible species experience more severe and frequent defoliation and have higher mortality than do non-susceptible species (Campbell and Sloan 1977a; Herrick and Gansner 1987; Quimby 1985, 1987).

Mortality can vary from stand-to-stand, even when stands have similar characteristics with mortality 80 to 100 percent in some stands (Campbell and Sloan 1977a, Gansner and Herrick 1984). Most mortality occurs during and after the initial outbreak (Twery 1991) with severe mortality along and behind an advancing outbreak front as the gypsy moth invades new areas (Gansner and Herrick 1984, Herrick and Gansner 1986, Twery and Gottschalk 1988). Subdominant trees typically have much higher mortality rates than dominant trees after heavy defoliation (Campbell 1979, Gansner and others 1993c. Quimby 1993). The most common response to canopy

gaps created by tree mortality is increased growth and density of existing understory woody plants (Collins 1961, Ehrenfeld 1980, Feicht and others 1993, Hix and others 1991, USDA Forest Service 1994f).

Drought may increase the severity of gypsy moth effects on trees (Bess and others 1947, Campbell and Sloan 1977a, Stephens and Hill 1971). Should severe drought occur with repeated years of defoliation, the cumulative impacts may increase mortality. Stress from disturbances, such as timber cutting or fire, and naturally occurring oak decline can also increase mortality.

Forest Condition—Seed and Mast Production.

Nuts, seeds, and fruits that serve as food for animals in the forest are called mast. Seed production by defoliated oak trees is reduced directly through consumption of oak flowers and young acorns by gypsy moth caterpillars, and indirectly by abortion of acorns and—in the years after defoliation–reduced initiation of flower buds. Significant mortality of oaks (more than 60 percent of basal area in a stand) must occur before acorn production is reduced significantly (Gottschalk 1990b).   Over the long term, an increase in soft mast, particularly berries, replaces the loss of hard mast such as acorns (Gottschalk 1990a), and mammals that usually eat acorns may start eating this soft mast.

Water Quality.

Defoliation by the gypsy moth may affect a number of characteristics of nearby water bodies, including temperature, flow rate and yield, sediment load, acidity levels, oxygen availability, nutrient concentration, and structural habitat for aquatic organisms. Defoliated riparian areas receive increased exposure to the sun. Increases in the amount of light penetrating stream surfaces and changes in water temperature can affect both plants and animals in the stream. Various factors influence stream temperature at a given point, including flow volume, hydraulic gradient, ground water discharge, degree of shading, and upstream conditions.

Actual changes to water temperature vary from site to site and depend in part upon the degree and duration of defoliation (USDA Forest Service 1994f). On a headwater stream under a dense tree canopy, light penetration increased from 5 to 18 percent to 73 percent after a "massive" gypsy moth outbreak in Rhode Island (Sheath and others 1986). Water temperature increased by 3.7 °C (6.7 °F) in early July, and algal growth in the streambed increased dramatically.

Defoliation by the gypsy moth has been shown to increase water yield (Corbett and Lynch 1987), in part due to fewer available leaves to transpire moisture from the soil (Twery 1991). Increased water yields from forested watersheds may produce beneficial results, such as creating more wet areas during summer, which might enhance habitat for amphibians. Conversely, increased stream discharge may have a destabilizing effect on herbivorous insects (Eagle 1993).

Sediment loads from forested land are usually low; however, increases in stream velocities due to increased water yield can lead to increased erosion, sedimentation, and turbidity. Timber cutting, exclusive of disturbances caused by road construction and log removal, usually has little if any effect on stream turbidity and sedimentation (Corbett and Lynch 1987). Therefore, gypsy moth defoliation would be unlikely to cause an increase in watershed erosion.

Whenever defoliation by the gypsy moth causes tree mortality in riparian areas, the structural habitat of streams may be altered by deposition of woody debris in affected streams. Debris dams may trap more organic material, lengthening the time it is available for ingestion by benthic invertebrates and leaf shredders, and allowing for more complete energy utilization. Large, woody materials also provide improved fisheries habitat (USDA Forest Service 1994f).

Defoliation by the gypsy moth may contribute to alterations in water chemistry and a reduction in the capacity to neutralize acids in some streams associated

with upland watersheds in the southern Appalachian region (USDA Forest Service 1994f). Defoliation temporarily produces conditions typical of winter, such as reduced acid-neutralizing capacity and increased acidity (Downey 1991). Acid-neutralizing capacity determines the concentrations of hydrogen and aluminum in solution, which at elevated levels are toxic to fish and other aquatic organisms. Acid-neutralizing capacity of streams increases seasonally, when deciduous leaves are present in the tree canopy.

Increased organic matter in streams from gypsy moth frass and leaf fragments, in combination with increased light penetrating the surface of the water, may lead to over-enrichment and result in excessive growth of algae and other microorganisms. This bloom could cause a reduction in oxygen available to other organisms in the stream. Large increases in fecal coliform and streptococci densities have been observed in streams where heavy gypsy moth defoliation has occurred (Corbett and Lynch 1987).

Defoliation is also suspected of causing increased nitrate mobility, which would allow nitrate to be lost from a site. Elevated concentrations of nitrate in streams have been associated with forest harvest (Vitousek and Melillo 1979) and defoliation by insects (Swank and others 1981, USDA Forest Service 1994f). Defoliation by the gypsy moth can accelerate the transfer of nutrients from vegetation to the soil surface; however, there is little evidence that these nutrients are lost from the site and enter adjacent water bodies to a significant degree (Eagle 1993, Grace 1986).

Soil Condition.
Gypsy moth defoliation probably increases the rate of decomposition of organic matter and decreases soil moisture content because of the greater penetration of sunlight increasing biological activity (Grace 1986, Tomblin 1994). These changes should result in short-term increases in biological productivity.

Microclimate.
The microclimate of defoliated areas is affected by rises in soil, leaf litter, and ambient air temperatures due to increased exposure to sunlight (Vaughan and Kasbohm 1993).

Risk to Human Health (Issue 1).

General.
People coming in contact with gypsy moth larvae may have skin irritation, resembling mosquito bites, with raised patches of skin approximately 0.25 to 0.5 inches in diameter. Some people may have itching persisting several days to 2 weeks and sufficiently severe to cause them to seek medical treatment. Heavy infestations or extreme outbreaks potentially cause eye and respiratory effects in some individuals. Heavy infestations are often considered a public nuisance, causing esthetic damage to the environment through tree defoliation which may induce stress or anxiety in some individuals.

Groups at Special Risk.
Young children are potentially at greater risk of effects from gypsy moth exposure perhaps because they spend more time outdoors than adults (Tuthill and others 1984, Aber and others 1982, Anderson and Furniss 1983).

Risk to Nontarget Organisms (Issue 2).

Mammals.
Fur reduces the risk of direct contact with gypsy moth hairs making skin irritation unlikely. Evidence of irritation to the eyes and or respiratory tract in mammalian wildlife species after direct contact with the gypsy moth is not found in the literature.

To determine the effects of a gypsy moth outbreak on a population of black bears (*Ursus americanus*), Vaughan and Kasbohm (1993) monitored the behavior of 54 radio-collared black bears in the Shenandoah National Park after a gypsy moth outbreak that caused widespread defoliation, hard mast failures, and tree mortality. The outbreak had no apparent effects on cub production or mortality rates of cubs or adults. In the fall, before the gypsy moth infestation, the bears ate mostly acorns. When acorns were no longer available due to defoliation, the bears switched to eating fruit, which had no apparent impact on the nutritional quality of their diets. Seventy-one percent of bear dens were in tree cavities, primarily in living oaks. Gypsy moth-induced mortality of den trees was high and, by the end of the study, 54 percent of the living oaks used as dens were dead. While no short-term effects were noted, Vaughan and Kasbohm (1993) speculated that the long-term adverse impact of defoliation on black bears may be a reduction in den sites, with natural replacement possibly requiring 50 years. Conversely, black bears will use upturned stumps of large dead trees as dens. These would be expected to increase as tree mortality increases.

Variations in acorn and other mast production are directly related to variations in populations of squirrels, mice, and other small mammals (Brooks and others 1998). Acorn crop size in the fall directly affects the population density of mice living in oak-dominated forests the following spring (McShea and Rappole 1992, McShea and Schwede 1993). A decrease in acorn production has been shown to decrease the population of white-footed mice, *Peromyscus leucopus* (Elkinton and others 1996, 2002).

White-tailed deer will migrate to areas that have not been defoliated. Nesting failures of grouse and turkey may increase. Bear, turkey, and bats may migrate to nondefoliated areas or less defoliated areas (USDA 1995).

Sample and others (1996) found no significant effects on the consumption of insects by Virginia big-eared bats in areas of high gypsy moth infestation and defoliation.

Birds.
Some species of birds appear to avoid the gypsy moth as a prey species (Smith 1985), perhaps because of larval hairs. Reported increases in nesting failures of various species of birds appear to be due to increased predation, increased weather stress, or both, which are associated with defoliation (Thurber and others 1994).

Gypsy moth infestations and subsequent defoliation may be beneficial to some species of birds, especially species that favor dead wood (snags) as a habitat (Bell and Whitmore 1997a, b; DeGraaf 1987; DeGraaf and Holland 1978; Showalter and Whitmore 2002). Available nesting and foraging resources increased for several bird species as a result of more snags, windfall, and shrub cover after defoliation, while there was no substantial impact from upper canopy defoliation on birds residing primarily in the forest canopy (Bell and Whitmore 1997a, b).

Cavity-nesting birds benefit indirectly from a gypsy moth outbreak (Showalter and Whitmore 2002). Bird density increased in plots with low to moderate defoliation (Thurber 1993). Species richness increased from 19 to 23 species per plot, with declines noted only for tree nesters and flycatchers on high-impact plots (Thurber 1993). Increases in low shrub and ground nesters, cavity nesters, low shrub and ground foragers, bark foragers, forest edge species, short-distance migrants, year-round residents, and woodpeckers were widespread, but most pronounced on moderate-impact plots. DeGraaf and Holland (1978) reported similar results, finding significantly fewer numbers of only 4 out of 36 bird species examined in heavily defoliated areas. No substantial effects on abundance of various species of birds in defoliated and nondefoliated stands were noted in central Pennsylvania over a 2-year period (DeGraaf 1987).

Terrestrial Invertebrates.

Some lepidopteran species may be adversely affected by gypsy moth outbreaks. Redman and Scriber (2000) examined the adverse effects of the gypsy moth on the northern tiger swallowtail butterfly, *Papilio canadensi*. Direct effects included 100 percent mortality in *Papilio* larvae exposed to leaves painted with gypsy moth body fluids, and 84 percent mortality in *Papilio* larvae fed leaves from aspen stands infested with gypsy moth larvae.

The potential adverse effects of gypsy moth outbreaks to Lepidoptera was also investigated in a study designed to compare lepidopteran populations in 50 acre plots in mixed oak, hickory, and pine forests in West Virginia (Sample and others 1996). Decreases in abundance and richness of larvae and adults from the family Arctiidae (tiger moths) were apparent in plots infested with gypsy moth larvae, compared with uncontaminated plots.

The impact of the gypsy moth is negative to only a small proportion of the lepidopteran community, primarily species that feed on oak and for which the larval development of the affected species and gypsy moth presumably coincide (Work and McCullough 2000). Although the study does not address the mechanism(s) by which the gypsy moths adversely affect the lepidopteran community, the investigators suggest they might include altered host plant quality, increases in natural enemies, or microclimate changes.

Some reports suggest that certain lepidopteran species respond positively to gypsy moth infestations. In 1981, the number of butterfly species was at a record high for the New Haven, Connecticut, area, despite the record number of acres defoliated by the gypsy moth that same year (Schweitzer 1988).

Fish.

Little information is available regarding the effects of gypsy moth infestations on fish populations. Defoliation by the gypsy moth can result in an increase in the pH and temperature of ambient water (Downey and others 1994, Webb and others 1995a). Trout, which are very sensitive to changes in pH and temperature, could be adversely affected by such changes (Downey and others 1994). No direct data are available on the biological effects of such changes due to gypsy moth defoliation (Webb and others 1995a).

Aquatic Invertebrates.

The rate of leaf breakdown in streams apparently increased due to gypsy moth defoliation, which might result in food deficits during spring for shredders, such as caddisflies, stoneflies, and some dipterans (Hutchens and Benfield 2000). The number of shredders collected, however, was greater in disturbed streams (i.e., streams in areas of gypsy moth defoliation) than in control streams.

Cumulative Effects of the Gypsy Moth.

Risk to Human Health (Issue 1).

The available data do not permit a definitive assessment of the effects of exposure to the gypsy moth over several seasons. Some individuals may become sensitized to the gypsy moth after repeated exposures over one or more seasons. Young children may be a group at special risk from effects of gypsy moth exposure but it is not clear whether children are more sensitive than adults to the effects of gypsy moth exposure or whether responses in children appear greater because children spend more time outdoors than with adults do.

Risk to Nontarget Organisms (Issue 2).

Effects due to the gypsy moth would be cumulative in situations of repeated outbreaks and defoliation in the same area. Repeated defoliation would lead to changes in forest condition that are characterized by increased tree mortality, stand structure and composition changes, a shift from production of hard to soft mast, and increased fire danger.

Habitats of wildlife species are altered more with each successive outbreak of the gypsy moth. Recolonization of species lost or displaced due to changes in habitat is possible; however, large areas of defoliation and frequent repeated defoliation do not favor recolonization by species with low dispersal capabilities.

Economic and recreational consequences that accumulate with repeated multiyear outbreaks include these: costs associated with annual cleanup; maintenance and replacement of trees that die; and loss of value from reduced growth and mortality of trees.

4.4 Consequences of *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*) (Alternatives 1, 2, 3)

See Appendixes F and M for detailed analysis of risks associated with *B.t.k*.

General Effects of *B.t.k.*

B.t.k. may indirectly help to maintain existing forest conditions, water quality, microclimate, and soil condition by delaying increases in gypsy moth populations, thereby protecting tree foliage.

Risk to Human Health (Issue 1).

General.

B.t.k. and its formulations may cause irritation to the skin, eyes, and respiratory tract; however, serious adverse health effects are improbable. Overt signs of systemic toxicity are not likely to be observed in any group–ground workers, aerial workers, or members of the general public–that is exposed to *B.t.k.* as the result of gypsy moth management programs conducted by the USDA (Appendix M). Throat irritation is the most frequently documented effect of *B.t.k.* in the scientific literature on human health (Appendixes F and M). Dermal and ocular irritations are observed at the extreme upper levels of exposure.

There is little indication that *B.t.k.* is associated with pathogenicity in humans and no indication of endocrine disruption or reproductive effects. Carcinogenic and mutagenic effects are not likely. Neither *B.t.k.* nor its commercial formulations are highly toxic or infectious (Appendixes F and M). Formulations of *B.t.k.* are likely to cause irritant effects to the skin, eyes, and respiratory tract; however, concerns about serious adverse health effects are not plausible. This risk characterization is consistent with the risk characterization in the previous USDA risk assessment (USDA 1995), as well as with more recent risk assessments conducted by the U.S. Environmental Protection Agency (EPA) and the World Health Organization, and the comprehensive review of *B.t.* published by Glare and O'Callaghan (2000).

Pretreatment with an influenza virus substantially increased mortality in mice exposed to various doses of *B.t.k* (Hernandez and others 2000). These results raise questions about the susceptibility of individuals who contact influenza or other viral respiratory infections prior to *B.t.k.* applications and have viral infections at the time of application. The enhancement of bacterial infections by a virus is not uncommon, and the enhancement of *B.t.k.* toxicity by a viral infection is, in some respects, not surprising. The relevance of this observation to public health cannot be completely assessed at this time. Several epidemiological studies have been conducted on the effects of *B.t.k.* on human populations, and none have reported viral enhancement. It is uncertain whether epidemiology studies would detect such an effect or whether such an effect is plausible under the anticipated exposure levels used in programs to control the gypsy moth. The viral enhancement of *B.t.k.* toxicity is likely to be an area of further study in the coming years.

Groups at Special Risk.

The available toxicity data give no indication that subgroups of the general population are likely to be remarkably sensitive to *B.t.k.* Nonetheless, *B.t.k.* formulations are complex mixtures and there is a

possibility that certain individuals may be allergic to one or more of the components in the formulations. The study by Hernandez and others (2000) also raises concern regarding the susceptibility of individuals with influenza or other viral respiratory infections to *B.t.k.* toxicity (Appendix F). See Appendixes F and M for detailed information.

Risk to Nontarget Organisms (Issue 2).

Mammals.

Adverse effects due to *B.t.k.* are unlikely in mammals (Appendixes F and M). Most inhalation studies do not suggest the potential for adverse effects, even at *B.t.k.* concentrations much greater than those likely to be encountered in the environment (Appendix F). Bats that feed almost exclusively on lepidopterans might be indirectly affected through a reduction in prey, as suggested by a study in West Virginia (Sample and others 1993a, b; Sample and Whitmore 1993). A 3-year study (1990-1992) conducted in West Virginia on food of the endangered big-eared bat revealed the greatest impact within 3 weeks of *B.t.k.* application due to reduction of prey species. Contrasting these studies, Sample and others (1996) showed that the moths on which bats feed were not affected by *B.t.k.* applications.

Birds.

Acute toxic effects are not likely in birds (Appendixes F and M). Due to the lack of toxicity of *B.t.k.* formulations, as well as of other *B.t.* strains, the U.S. EPA did not require chronic or reproductive toxicity studies in birds (Appendix F). This apparent lack of toxicity is supported by numerous field studies in birds. *B.t.k.* applied at rates sufficient to decrease the number of caterpillars had no substantial adverse effects on most bird species (Rodenhouse and Holmes 1992, Nagy and Smith 1997, Sopuck and others 2002). However, a study showed a significant decline in three species of insectivorous birds (black throated

green warbler, eastern tufted titmouse, and yellow-billed cuckoo), but they fully recovered within 3 years (Strazanac and Butler 2005).

A field study that included intensive searches of plots in sprayed and unsprayed areas revealed no differences in the numbers of songbird broods between the two areas for any of the species examined (Sopuck and others 2002). A reduction of lepidopteran larvae due to *B.t.k.* application appeared to have only minimal effects on reproduction in hooded warblers (Nagy and Smith 1997). The reduction in numbers of birds in an area observed in some species was considered indirect and attributed to alterations in the availability of prey rather than to the direct toxicity of *B.t.k.* (Gaddis 1987; Gaddis and Corkran 1986; Norton and others 2001).

Terrestrial Invertebrates.

B.t.k. is toxic to several species of target and nontarget Lepidoptera. The larvae of the Karner Blue Butterfly (a Federally listed endangered species), two species of swallowtail butterflies, a promethean moth, the cinnabar moth and various species of Nymphalidae, Lasiocampidae, and Saturniidae are susceptible to *B.t.k.* (Glare and O'Callaghan 2000)

Permanent changes in nontarget caterpillar populations do not appear likely as a result of gypsy moth management projects. An exception might occur in certain habitat types that support small isolated populations of lepidopterans that are highly susceptible to *B.t.k.* If unaffected individuals of the same species are unlikely to, or physically cannot, move from the treated into the untreated area, then one application of *B.t.k.* will have an effect on the ability of those populations to recover. These effects are limited to spring caterpillars that are present during *B.t.k.* treatments (Strazanac and Butler 2005). Full recovery of nontarget spring caterpillars occurred within 1 to 2 years after the treatment (Strazanac and Butler 2005).

In Oregon, Miller (1990) observed reductions in both types and numbers of nontarget caterpillars after three applications of *B.t.k.* The reductions persisted for 1 year after treatment but not for 2 years. In another study (Carter and others 1995), a second application of *B.t.k.* did not increase mortality of five species of Lepidopterans over that caused by one application. The species tested were moderately resistant to *B.t.k.* and had mortality rates below 50 percent after the first application.

While some nontarget lepidopteran species appear to be as sensitive to *B.t.k.*, most studies indicate that effects in other terrestrial insects are likely to be of minor significance (Appendix F). There is relatively little information regarding the toxicity of *B.t.k.* or *B.t.k.* formulations to terrestrial invertebrates other than insects. For some Lepidoptera, sensitivity to *B.t.k.* is highly dependent on their developmental stage. This is particularly evident for the cinnabar moth, where late instar larvae are very sensitive to *B.t.k.* and early instar larvae are very tolerant to *B.t.k.* (James and others 1993).

The variability in the response of nontarget Lepidoptera to *B.t.k.* is also illustrated in a recent field study in which a *B.t.k.* formulation was applied to two forests (dominated by oak, hickory, and maple trees) over a 2-year period, at an application rate of 40 BIU/acre (Rastall and others 2003). Researchers monitored nontarget lepidopteran populations in the 2 years prior to application as well as over the 2-year period in which *B.t.k.* was applied. The response of nontarget Lepidoptera varied substantially among different species. Larvae of three lepidopteran species significantly decreased in treatment years: *Lambdina fervidaria* (geometrid), *Heterocampa guttivitta* (notodontid), and *Achatia distincta* (noctuid). For 19 other species, larval counts were significantly higher in treatment years as were the total number of noctuids combined and the total number of all nontarget lepidopteran species combined. The Karner Blue

Butterfly is susceptible to *B.t.k.,* although the larval generation at risk may vary from year to year (Herms and others 1997).

Some predators and parasitoids may be affected indirectly by *B.t.k.* because of the loss of gypsy moth caterpillars that they parasitize or eat. The more specific the parasites and predators are for lepidopterans affected by *B.t.k.,* the greater the chance of an effect. For example, populations of parasitoid tachinid flies and Braconidae wasps and Pentatomidae stinkbugs declined after application of *B.t.k.* (then recovered by the second year), but generalist predators did not decline (Strazanac and Butler 2005).

Fish.
The U.S. EPA classifies *B.t.k.* as virtually nontoxic to fish (Appendix F). This assessment is consistent with the bulk of experimental studies reporting few adverse effects in fish exposed to *B.t.k.* concentrations that exceed environmental concentrations associated with USDA programs (Buckner and others 1975, Otvos and Vanderveen 1993).

Aquatic Invertebrates.
The effects of *B.t.k.* on aquatic invertebrates is examined in standard laboratory studies and in numerous field studies. *B.t.k.* may be lethal to certain aquatic invertebrates, like *Daphnia magna*, at concentrations high enough to cause decreases in dissolved oxygen or increased biological oxygen demand (Young 1990). Most aquatic invertebrates seem relatively tolerant to *B.t.k.* (Appendix F, Section 4.1.3.3). This assessment is supported by several field studies that failed to note effects in most species after exposures that substantially exceed expected environmental concentrations (Kreutzweiser and others 1992, 1993, 1994).

Cumulative Effects of *B.t.k.*

Risk to Human Health (Issue 1).

Given the reversible nature of the irritant effects of *B.t.k.* and the low risks for serious health effects, cumulative human health effects from spray programs conducted over several years are not expected. Mating disruption with disparlure will most likely be the only other treatment used in the same spray blocks with *B.t.k.* However, *B.t.k.* is used to treat gypsy moth larvae, and mating disruption is used against gypsy moth adults, and they are applied weeks apart. These treatments also have different modes of action, and there are no known cumulative effects between the treatments.

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

There is no known documented evidence of a subgroup of individuals who are more sensitive than most members of the general public to *B.t.k.* formulations (Appendix F).

Risk to Nontarget Organisms (Issue 2).

Many studies indicate that *B.t.k.* lasts about a week in the environment. Repeated treatments of areas with *B.t.k.* could potentially impact some species of spring-feeding butterfly and moth caterpillars. Since *B.t.k.* is not used in the same spray blocks with other treatments that could affect nontarget organisms, there is no cumulative effect between different treatments and *B.t.k.* on spring-feeding caterpillars.

4.5 Consequences of Diflubenzuron (Alternatives 1, 2, 3).

See Appendixes I and M for detailed analysis of risks associated with diflubenzuron.

General Effects of Diflubenzuron.

Diflubenzuron may indirectly help to maintain existing forest conditions, water quality, microclimate, and soil condition by delaying increases in gypsy moth populations, thereby protecting tree foliage.

Risk to Human Health (Issue 1).

General.

Diflubenzuron causes the formation of methemoglobin, a form of hemoglobin incapable of oxygen transport, normally present in the blood in small amounts. Methemoglobinemia, the formation of excess methemoglobin, is the primary toxic effect of diflubenzuron in every species of animal tested, regardless of the route or duration of exposure. While effects on the blood are well documented, there is little indication that diflubenzuron causes other specific forms of toxicity. Diflubenzuron does not appear to be neurotoxic nor immunotoxic, does not appear to affect endocrine function in laboratory mammals, and is not a carcinogen. Additionally, diflubenzuron does not appear to cause birth defects or to affect reproductive processes. Numerous studies regarding the subchronic and chronic toxicity of diflubenzuron in laboratory animals indicate that methemoglobinemia is the most consistent clinical symptom indicative of toxicity. Diflubenzuron can be absorbed via the skin in sufficient amounts to cause hematological effects, that is, methemoglobinemia and sulfhemoglobinemia. Nonetheless, the dermal exposure concentrations necessary to induce these hematological effects are higher than the oral exposure dosage necessary to cause the same effects.

Diflubenzuron rapidly dissipates from vegetation and is broken down by sunlight; in the environment the compound degrades to 4-chloroaniline, which the EPA considers a potential carcinogen. This is the only identified potential carcinogen associated with any of the agents to control gypsy moth. The compound is not expected to be present in significant amounts during application since 4-chloroaniline does not form during application. The scenario of greatest concern involving 4-chloroaniline is a cancer risk from drinking contaminated water. This risk would be most plausible in areas with sandy soil and annual rainfall rates ranging from about 50 to 250 inches. The estimate of the hazard quotient for the consumption of water contaminated with 4-chloroaniline and based on a cancer risk of 1 in 1 million is 0.09, which is 10 times lower than the level of concern.

None of the hazard quotients for diflubenzuron reaches a level of concern at the highest application rate used in USDA programs (Appendix I). Since many of the exposure assessments overestimate exposure, and because the dose-response assessment is based on similarly protective assumptions, there is no basis for asserting that this use of diflubenzuron poses a hazard to human health (Appendix I).

Groups at Special Risk.

Some individuals have congenital methemoglobinemia and may be at increased risk of adverse effects to compounds that induce methemoglobinemia (Barretto and others 1984). Infants less than 3 months old have lower levels of methemoglobin (cytochrome b5) reductase and higher levels of methemoglobin (1.32 percent), compared with older children or adults (Centa and others 1985, Khakoo and others 1993, Nilsson and others 1990). Some infants with an intolerance to cow's milk or soy protein exhibit methemoglobinemia (Murray and Christie 1993, Wirth and Vogel 1988). These infants would be at increased risk if exposed to any materials contaminated with diflubenzuron or any compound that induces methemoglobinemia.

Individuals with poor diets might be vulnerable to some chemicals. Based on a study in rats, iron deficiency leads to anemia but does not influence methemoglobin reductase activity (Hagler and others 1981). Thus, although individuals with poor nutritional status are generally a group for which there is particular concern, the available information does not support an increased risk for these individuals with respect to diflubenzuron exposure.

Risk to Nontarget Organisms (Issue 2).

Mammals.

The available field studies indicate no substantial impacts on mammalian wildlife from applications of diflubenzuron. Applications of 60 to 280 g a.i./ha (grams active ingredient per hectare) or 0.85 to 4 oz a.i./acre (ounces of active ingredient per acre) had no detectable adverse effects on the abundance of, or reproduction in moles, field mice, and shrews (O'Connor and Moore 1975; Henderson and others 1977). Small mammals increased in abundance on a plot receiving 280 g a.i./ha compared with a control plot (Henderson and others 1977). The adverse effects that diflubenzuron might have on bot flies, a parasite of small and large mammals alike, was suggested as a possible explanation.

A field study reported no effect on body measurements, weight, or fat content in populations of mice in areas treated with diflubenzuron (Seidel and Whitmore 1995). Mice in the treated areas did consume less lepidopteran prey, but total food consumption was not significantly different between treated and untreated plots.

Birds.

The acute toxicity of diflubenzuron to birds appears generally low. The lack of direct effects on birds is supported by several field studies summarized in Appendix I. Effects secondary to a reduction in lepidopteran prey may include increased foraging

range (Cooper and others 1990), relocation (Sample and others 1993a, b; Sample and Whitmore 1993) and lower body fat (Whitmore and others 1993).

Terrestrial Invertebrates.
Arthropods, a large group of invertebrates including insects, crustaceans, spiders, mites, and centipedes, are most sensitive to diflubenzuron. Most of these organisms use chitin as a major component of their exoskeleton (outer body shell). Diflubenzuron is an effective insecticide because it inhibits the formation of chitin, disrupting normal growth and development. Both terrestrial and aquatic arthropods are affected, though some substantial differences in sensitivity are apparent.

Invertebrates lacking exoskeletons, such as earthworms and snails, do not utilize chitin, and diflubenzuron is relatively nontoxic to these species (Appendix I). Species that are most sensitive to diflubenzuron include lepidopteran and beetle larvae, grasshoppers, and other chewing herbivorous insects (Berry and others 1993, Butler 1993, Butler and others 1997a, Elliott and Iyer 1982, Jepson and Yemane 1991, Kumar and others 1994, Sample and others 1993a, Sinha and others 1990, Redfern and others 1980). Species that are relatively tolerant to diflubenzuron include flies, parasitic wasps (on insect eggs), adult beetles, and sucking insects (Ables and others 1975, Broadbent and Pree 1984, Brown and Respicio 1981, Bull and Coleman 1985, De Clercq and others 1995, Delbeke and others 1997, Gordon and Cornect 1986, Keever and others 1977, Martinat and others 1988, Webb and others 1989, Zacarias and others 1998, Zungoli and others 1983).

The U.S. EPA uses the honey bee as the standard test species to classify the toxicity of pesticides to nontarget terrestrial invertebrates. Based on early acute oral and contact toxicity studies in honey bees (Atkins and others 1974, Stevenson 1978), the U.S. EPA (1997) classifies diflubenzuron as practically nontoxic to honey bees. Several other laboratory toxicity studies

also indicate diflubenzuron is not particularly toxic to bees (Chandel and Gupta 1992, Elliott and Iyer 1982, Gijswijt 1978, Kuijpers 1989, Nation and others 1986, Yu and others 1984). This conclusion is supported by several field studies conducted at application rates comparable to, or substantially higher than, those used to control the gypsy moth (Buckner and others 1975, Emmett and Archer 1980, Matthenius 1975, Schroeder 1978, Schroeder and others 1980). Additionally, no detectable amounts of diflubenzuron were found in honey bees in areas treated with diflubenzuron (Cochran and Poling 1995).

Fish.
Based on the available information, the U.S. EPA (1997) classifies acute exposure to diflubenzuron as "practically nontoxic" to fish. The 96-hour LC_{50} values range from greater than 25 milligrams per Liter (mg/L) (the value for yellow perch reported by Johnson and Finley 1980) to greater than 500 mg/L (the value for fathead minnow reported by Reiner and Parke 1975). In addition, no effects were seen in longer-term studies at concentrations up to 100 parts per billion (ppb) (Cannon and Krize 1976) or in two-generation reproduction studies at concentrations of up to 50 ppb (Livingston and Koenig 1977).

Indirect effects on fish are plausible based on a decrease in invertebrate populations as demonstrated in studies in which concentrations as low as 2.5 ppb resulted in decreased growth of fish in littoral enclosures (populations of fish placed in enclosures along the shore of a body of water and monitored) (Moffett 1995, Tanner and Moffett 1995). The reduced growth observed in these studies is attributed to a reduction in macroinvertebrates, a fish food source.

None of the field studies summarized in Appendix I note any adverse effects on fish at application rates comparable to or greater than those used in the control of the gypsy moth. A study by Colwell and Schaefer

(1980) did note a shift in the diet of fish (secondary to changes in food availability) but no effect on growth rates or general condition of the fish.

Aquatic Invertebrates.
Because diflubenzuron inhibits the synthesis of chitin, crustaceans are the aquatic invertebrates most sensitive to diflubenzuron. Many bioassays, both acute and chronic, have been conducted on *Daphnia magna* (Hansen and Garton 1982, Kuijpers 1988, Majori and others 1984, Surprenant 1988) as well as a related species, *Ceriodaphnia dubia* (Hall 1986). As detailed further in the dose-response assessment (Appendix I), these organisms are among the most sensitive to diflubenzuron (Hall 1986, Hansen and Garton 1982). Several other crustacean species appear to be about as sensitive as or only somewhat less sensitive to diflubenzuron than daphnids are (Appendix I). Small crustaceans that consume algae and serve as a food source for fish, such as *Daphnia* species, appear to be the most sensitive to diflubenzuron, while larger insect species, such as backswimmers and scavenger beetles, are much less sensitive. Other aquatic invertebrates, crustaceans, and small- to medium-sized aquatic insect larvae appear to have intermediate sensitivities.

Snails, aquatic worms, and bivalves were not affected by exposure to diflubenzuron (Hansen and Garton 1982; Surprenant 1989).

Field studies on the effects of diflubenzuron on aquatic invertebrates reinforce the standard toxicity studies, indicating diflubenzuron will impact invertebrate populations. Several of these studies, however, were conducted at application rates substantially higher than those used to control the gypsy moth. Many of the studies in which severe adverse effects were observed in aquatic invertebrate populations involved multiple applications at rates between about 110 g/ha and 560 g/ha (Ali and Mulla 1978a, b; Ali and others 1988; McAlonan 1975). Concentrations in this range are substantially higher than the application rate of 17.5 g/

ha that is likely to be encountered in USDA programs. Similarly, other field studies involve direct applications to open water, a treatment method that is not part of USDA program activities, and which resulted in concentrations of diflubenzuron in water in the range of 10 ppb (Apperson and others 1977, Boyle and others 1996, Colwell and Schaefer 1980, Lahr and others 2000, Sundaram and others 1991).

Diflubenzuron reduces numbers of stream invertebrates that process detritus; however, field studies have shown no decline in detrital decomposition rates (Swift and others 1988). The populations of some invertebrates that feed on algae are reduced by diflubenzuron. An increase in algae could occur after the loss of algal herbivores, however, this has not been observed in field studies.

Field studies using lower application rates that are more typical of USDA gypsy moth management programs noted some effects on freshwater invertebrates, particularly smaller crustaceans (Farlow 1976; Griffith and others 1996, 2000; Hurd and others 1996; Reardon 1995). The effects were much less severe than those seen at higher application rates. See Section 4.4 of Appendix I for further discussion.

Cumulative Effects of Diflubenzuron.

Risk to Human Health (Issue 1).
Diflubenzuron is not likely to be used with other treatments at the same site, so no cumulative effects with other treatments are likely. Multiple applications at lower rates per application result in lower associated risks than with a single application at the maximum approved rate.

Diflubenzuron and tebufenozide could have a cumulative effect on methemoglobinemia. USDA gypsy moth management programs do not use these two chemicals together in the same area at the same time. Exposure to other methemoglobinemia-inducing compounds in the environment may contribute to a

cumulative effect. Individuals exposed to combustion smoke or carbon monoxide (agents causing oxidative damage to blood) may be at increased risk of developing methemoglobinemia. Individuals exposed to high levels of nitrates, either in air or in water, demonstrate increased levels of methemoglobin and may be at increased risk with exposure to compounds such as diflubenzuron.

Some infants with congenital methemoglobinemia and an intolerance to cow's milk or soy protein exhibit methemoglobinemia. These infants would be at increased risk if exposed to any materials contaminated with diflubenzuron.

Risk to Nontarget Organisms (Issue 2).

Diflubenzuron is generally not used in conjunction with other treatments; however, diflubenzuron might be applied to the same area in multiple years for eradication projects. In that case, diflubenzuron might have a cumulative effect on nontarget invertebrates, such as caterpillars of moths and butterflies, grasshoppers, parasitic wasps, aquatic insects, bottom dwelling crustaceans, and immature free-floating crustaceans. Diflubenzuron applications as used in USDA treatment projects will otherwise have no cumulative effects.

4.6 Consequences of Disparlure (as Used in Mating Disruption and Mass Trapping) (Alternatives 1, 2, 3).

See Appendixes H and M for detailed analysis of risks associated with disparlure as used in mating disruption and mass trapping.

In mating disruption, a medium is impregnated with disparlure for timed release and formulated for aerial application over the project area. The objective is to flood the area with pheromone, thereby impeding the male moth's ability to find and mate with female moths. Also, in mass trapping, a solid medium is impregnated with disparlure, formulated for timed release, and deployed in small "delta" or large capacity "milk carton" traps. The traps are deployed across the treatment area to attract and capture male moths, thereby preventing them from finding and mating with female moths. The delta and milk carton traps are also used in detection surveys for gypsy moth.

General Effects of Disparlure.

Disparlure is specific to the gypsy moth and may indirectly help to maintain existing forest conditions, water quality, microclimate, and soil condition by delaying increases in gypsy moth populations, thereby protecting tree foliage.

Risk to Human Health (Issue 1).

General.

Insect sex pheromones are chemicals produced by insects for communication between the sexes of the same species. Insect pheromones are generally regarded as nontoxic to mammals and are commonly employed in very low concentrations. Consequently, the U.S. EPA requires less rigorous testing of these products than is required of chemical insecticides. Results of acute exposure studies for oral, dermal, ocular, and inhalation exposure to disparlure reveal no adverse effects. Based on the results of studies on disparlure itself (i.e., the active ingredient), acute exposure to disparlure exhibits very low toxicity to mammals.

No studies were identified investigating the effects of chronic exposure of mammals to disparlure or investigating the effects of disparlure on the nervous, immune, reproductive or endocrine systems of mammals. The carcinogenic potential of disparlure has not been assessed, though a single study focusing on mutagenicity revealed no indication that disparlure is mutagenic. No information is available regarding the kinetics and metabolism of disparlure in mammals; available literature does not document absorption

of disparlure following dermal, oral, or inhalation exposure. A case report of an occupational exposure indicates that disparlure may persist in humans for years (Cameron 1981, 1983).

Although studies on the acute toxicity of disparlure have been conducted in laboratory animals, the lack of either subchronic or chronic toxicity data precludes a quantitative characterization of risk.

Groups at Special Risk.
The toxic effects of disparlure, if any, have not been identified. Consequently, groups at special risk cannot be characterized.

Risk to Nontarget Organisms (Issue 2).

Mammals.
Results of acute toxicity studies for oral, dermal, ocular, and inhalation exposure to disparlure demonstrate very low toxicity to mammals. Information is not available regarding chronic toxicity, and no field studies exist assessing the impact of disparlure on mammals.

Birds.
There is no evidence that birds are affected by USDA treatment projects using disparlure.

Terrestrial Invertebrates.
Disparlure does not attract any other insect found in North America.

Fish.
Limited data are available regarding the toxicity of disparlure to aquatic animals. A major issue in the interpretation of the aquatic toxicity data on disparlure involves the solubility of disparlure in water. While no measured values are available, estimates based on quantitative structure-activity relationships developed by the U.S. EPA suggest that the solubility of disparlure in water is in the range of 0.0019 to 0.0028 mg/L (Appendix H). No risks to fish can be identified under foreseeable circumstances in the use of disparlure formulations.

Aquatic Invertebrates.
As with fish, disparlure does not appear to pose a risk to aquatic invertebrates due to inherent toxicity. At the limit of the solubility of disparlure in water, there is no indication that toxic effects are likely in any aquatic species (Appendix H). Based on the variability in the experimental data as well as the range of application rates used in USDA programs, HQs would vary from about 0.15 to about 0.37 below the level of concern by factors of about 3 to 10. This risk characterization applies to accidental application of disparlure to a body of water 1 meter deep. The HQ will vary with the depth of the water. Since the calculations are based on a 1-meter-deep body of standing water, the HQ would be a factor of 10 lower in a 10-meter-deep body of standing water and a factor of 10 higher in a 0.1-meter-deep body of standing water. In actual field applications using Disrupt II flakes, water bodies such as lakes and rivers are never directly treated with flakes, and levels of exposure in moving water would be magnitudes lower than the calculated static level, providing an even greater margin between exposure and potential toxicity. Further, control tests using the untreated carrier products (small plastic flakes) showed no toxicity.

In summary, the application of disparlure in mating disruption is unlikely to affect aquatic invertebrates.

Cumulative Effects of Disparlure.

Risk to Human Health (Issue 1).
Since disparlure seems to persist in humans, repeated exposures of disparlure will attract the gypsy moth. No information is available on the interaction of disparlure with other control agents or other chemicals usually found in the environment.

Risk to Nontarget Organisms (Issue 2).

Since disparlure attracts only the gypsy moth in North America, no cumulative effects are expected on nontarget organisms.

4.7 Consequences of Dichlorvos (as Used in Mass Trapping) (Alternatives 1, 2, 3).

See Appendixes K and M for detailed analysis of risks associated with the use of dichlorvos in mass trapping. Appendix A provides an in-depth discussion of how dichlorvos is used in mass trapping. Dichlorvos is *not* a distinct treatment in the USDA gypsy moth management program. It is simply an insecticide (formulated in a vinyl strip as a killing agent) used in the large-capacity milk carton trap, which can be deployed for mass trapping of male gypsy moths in a project area. This same kind of milk carton traps (with dichlorvos) are also used in gypsy moth surveys. Without this insecticide in the traps, the male gypsy moths that are attracted to traps (by disparlure) would simply fly back out.

Milk carton traps with dichlorvos have not been used for mass trapping since 1997 and only twice between 1993 and 1997, where no more then 50 acres were treated. Each year for surveys APHIS deploys approximately 19,000 milk carton traps with dichlorvos pest strips. The Forest Service's slow-the-spread strategy also uses milk carton traps for surveys.

General Effects of Dichlorvos.

Because dichlorvos is used inside traps, no effect on human health and nontarget organisms is expected. A person or animal would have to deliberately eat the resin strip. In the entire history of USDA use of traps containing dichlorvos, such an accidental or deliberate action has not been encountered.

Risk to Human Health (Issue 1).

General.

Dichlorvos is readily absorbed into the body of mammals via all routes of exposure, and is rapidly metabolized and eliminated. Generally, the systemic effects observed after oral, inhalation, or dermal exposure of humans or laboratory animals to dichlorvos result from the inhibition of acetylcholinesterase (AChE). The enclosed nature of milk carton traps containing dichlorvos minimizes the chance that people will come into contact with it. In a risk assessment of the carcinogenic and mutagenic potential of diclorvos, EPA decided "The carcinogenicity potential of Diclorovos has been classified as 'suggestive' under the 1999 Draft Agency Cancer Guidelines and no quantitative assessment of cancer risk is required." (Section 3.1.10 of Appendix K).

Exposure of both workers and members of the general public should be negligible in most cases. Workers taking prudent steps to limit both dermal and inhalation exposures can minimize the likelihood of exposure to dichlorvos. Similarly, exposure of the general public to substantial amounts of dichlorvos is unlikely. The dichlorvos is contained within a PVC strip to ensure the active ingredient is released slowly over time. The strip, in turn, is placed within a trap and the trap is placed so that it will not be accessed except in the case of intentional tampering or trap monitoring.

The greatest risks for workers are associated with inhalation exposures from assembling the traps in enclosed and poorly ventilated spaces, or while transporting the traps in the passenger compartments of vehicles. These risks are readily avoided. Dermal exposures are usually at lower levels than inhalation exposures.

All of the exposure scenarios for members of the general public described in Appendix K are accidental. Should a child come into contact with a dichlorvos strip, both dermal and oral exposures (if a child ate the

strip) could substantially exceed a level of concern. See Appendix K for additional dichlorvos information and risk assessment scenarios.

Groups at Special Risk.

Children are of primary concern as identified in the risk assessment (Appendix K). As noted above, imprudent handling of a dichlorvos-impregnated strip would most likely involve a child. Additionally, very young children (infants less than 6 months old) may be at special risk because of their incompletely developed AChE systems and immature livers (ATSDR 1993).

Several other groups may be at special risk to all cholinesterase-inhibiting compounds, including dichlorvos. A small proportion of the population has an atypical variant of plasma cholinesterase that may make them more susceptible to effects when exposed to dichlorvos and other AChE inhibitors. Other groups known to have low plasma AChE levels are long-distance runners, women in early stages of pregnancy, women using birth control pills, individuals with advanced liver disease, alcoholics, individuals with poor nutritional status, and individuals with skin diseases. Asthmatics may also be at special risk because dichlorvos may induce or exacerbate respiratory distress (ATSDR 1993).

Risk to Nontarget Organisms (Issue 2).

Exposure would be accidental since dichlorvos is used inside traps.

Mammals.

The principal adverse effects of dichlorvos exposure are directly related to inhibition of cholinesterase. In the USDA program for the control of the gypsy moth, the use of milk carton traps employing slow release of dichlorvos from PVC strips essentially precludes rapid exposures to high doses of dichlorvos.

Birds.

No published data is available concerning the acute toxicity of dichlorvos encased in PVC resin to birds.

Terrestrial Invertebrates.

The only terrestrial invertebrates likely to come into close contact with the dichlorvos strip are male gypsy moths attracted by the disparlure in the trap, or carnivorous wasps and hornets that may enter the trap to feed on dead and dying gypsy moths.

Fish.

There is no indication fish are likely to be adversely affected by dichlorvos as used in PVC strips (Section 4.4.3.1, Appendix K). However, dichlorvos itself is classified as highly toxic to both freshwater and estuarine fish (U.S. EPA 1999a). See Appendix K for comprehensive information.

Aquatic Invertebrates.

Based on the same conservative exposure assessment used for both fish and terrestrial vertebrates, some sensitive aquatic invertebrates could be adversely affected by dichlorvos contamination of water if a trap is intentionally thrown into water. As in the other exposure assessments developed in Appendix K involving contaminated water, this exposure scenario should be regarded as an extremely rare accident rather than routine. Under normal circumstances, water contamination from dichlorvos strips is negligible and consistent with the conclusions reached by U.S. EPA (1999a).

Cumulative Effects of Dichlorvos.

Risk to Human Health (Issue 1).

The only substantial exposures to the general public would occur from repeated tampering with traps containing dichlorvos. No such incidents have been reported, despite the long use of dichlorvos in traps for the gypsy moth and other species.

Workers may be exposed repeatedly to dichlorvos if they are involved in the assembly and placement of traps over a period of several weeks. No data exists regarding the effects of exposure to dichlorvos in combination with exposure to the other agents used to control the gypsy moth or to the gypsy moth itself. Inhibition of AChE is the most sensitive effect of dichlorvos; this effect is not associated with exposure to the other control agents or to the gypsy moth. Therefore, there is no plausible basis for assuming that the effects of exposure to dichlorvos and any or all of the other control agents or the gypsy moth are additive.

Risk to Nontarget Organisms (Issue 2).

Experience with traps used in mass trapping and survey programs shows that there are no cumulative effects on nontarget organisms even over years of use.

4.8 Consequences of Gypchek (Alternatives 1, 2, 3).

See Appendixes G and M for detailed analysis of risks associated with Gypchek.

General Effects of Gypchek.

Gypchek may indirectly help to maintain existing forest condition, water quality, microclimate, and soil condition by delaying increases in gypsy moth populations, thereby protecting tree foliage.

Risk to Human Health (Issue 1).

General.

According to Appendix G, there is no plausible risk to either workers or members of the general public from the use of Gypchek to control the gypsy moth.

Groups at Special Risk.

No groups at special risk are identified. Some individuals may be allergic to gypsy moth parts found in Gypchek.

Risk to Nontarget Organisms (Issue 2).

Mammals.

Except for eye irritation, there is little indication that NPV or the Gypchek formulation of NPV has any effect in mammals, even at extremely high levels of exposure. One study specifically focused on wildlife conducted by Lautenschlager and others (1977), exposed mice, short-tailed shrews, and opossums to various forms of NPV (gypsy moth larvae infected with NPV, a purified formulation of NPV, and a spray preparation of NPV). Based on gross observations, as well as necropsy and microscopic examination of several different tissues, no effects were seen in any of the species.

Birds.

Few studies are available on birds, and the results of these studies are essentially identical to those on mammals. The studies indicate exposures to NPV at levels that are substantially higher than those likely to occur in the environment are not associated with any adverse effects (Podgwaite and Galipeau 1978, Lautenschlager and others 1976).

Terrestrial Invertebrates.

Barber and others (1993) found no indication that NPV is pathogenic to any insect species except the gypsy moth. No adverse effects were observed in any species tested. Additionally, a recent field study noted no effects in nontarget insects following the application of Gypchek (Rastall and others 2003). There is no indication that adverse effects are caused in nontarget insects at any level of exposure.

Fish.

Two studies are available on the toxicity of NPV to fish (Moore 1977, Kreutzweiser and others 1997). The results of both studies show no toxicity in rainbow trout, no effects on mortality, behavior, or growth rate, and no viable NPV detected in the stomach or intestinal tract.

Aquatic Invertebrates.

No effects on mortality or reproduction were observed over exposure periods of up to 4 weeks (Streams 1976).

Cumulative Effects of Gypchek.

Risk to Human Health (Issue 1).

Exposure to both the gypsy moth caterpillars and Gypchek could be additive, although there are no data showing this occurs and Gypchek treatments would eliminate the caterpillars.

Risk to Nontarget Organisms (Issue 2).

Since Gypchek is specific to the gypsy moth, no cumulative effects are expected for nontarget organisms.

4.9 Consequences of Tebufenozide (Alternatives 2 and 3).

See Appendixes J and M for detailed analysis of risks associated with tebufenozide.

The use of tebufenozide to manage the gypsy moth may adversely affect nontarget Lepidoptera. There is little indication that humans or other wildlife species will be adversely affected under normal conditions of use, even at the highest application rate (see the full analysis of tebufenozide in Appendix J). *Table 4-2* provides hazard quotients (HQ) for tebufenozide and the other treatments and gypsy moth.

General Effects of Tebufenozide.

Tebufenozide may indirectly help to maintain existing forest conditions, microclimate, and soil condition by delaying increases in gypsy moth populations, thereby protecting tree foliage. Although tebufenozide is not highly mobile in soil, it may be transported by percolation, sedimentation, or runoff from soil to ambient water. Tebufenozide would not be sprayed over water or areas where surface water is present, and buffers will be maintained around these areas. See Appendix J for additional information on tebufenozide and water quality.

Risk to Human Health (Issue 1).

General.

A relatively detailed and consistent series of studies in mice, rats, and dogs indicates that the primary mechanism of tebufenozide toxicity in mammals involves effects on the blood, specifically the formation of methemoglobin. Tebufenozide does not appear to be carcinogenic and does not appear to cause birth defects. Nonetheless, the compound is associated with adverse reproductive effects in experimental mammals. Tebufenozide itself does not seem to be irritating to the skin or eyes. As discussed in the exposure assessment in Appendix J, dermal absorption is the primary route of exposure for workers. Data regarding the dermal absorption kinetics of tebufenozide are not available in the published or unpublished literature. Potential inhalation toxicity of the compound is not of substantial concern in the risk assessment in Appendix J.

At the maximum application rate, two applications at 0.12 lb (pounds) a.i./acre spaced 3 days apart, there is little indication that adverse effects on human health are likely. The risk assessment at Appendix J suggests, however; that two applications at 0.08 lb a.i./acre or more should be avoided in areas where members of the general public might consume contaminated fruits or other contaminated vegetation.

Groups at Special Risk.

Individuals born with a form of congenital methemoglobinemia may be at increased risk of adverse effects to compounds like tebufenozide that induce methemoglobinemia (Centa and others 1985, Das Gupta and others 1980). Some infants with an intolerance to cow's milk or soy protein exhibit methemoglobinemia. Infants less than 3 months old have lower levels of methemoglobin (cytochrome b5) reductase and higher levels of methemoglobin (1.32 percent) in comparison with older children or adults (Centa and others 1985, Smith 1996). A similar pattern is seen in many species of mammals (Lo and Agar 1986).

Risk to Nontarget Organisms (Issue 2).

Under normal conditions of use at the highest anticipated application rate, no effects are expected in any group of organisms: vertebrates, invertebrates, or plants.

Mammals.

everal standard toxicity studies in experimental mammals were conducted as part of the registration process for tebufenozide. The most sensitive effect in several species of experimental mammals involves effects on the blood, specifically the formation of methemoglobin.

The acute toxicity of tebufenozide is relatively low, with an oral LD_{50} greater than 5,000 mg/kg. The subchronic and chronic toxicity studies on tebufenozide were conducted in dogs, mice, and rats, with the most sensitive effects involving changes to the blood. There is no apparent dose-duration relationship for tebufenozide; short-term exposures are likely to lead to changes in the blood comparable to those observed following longer-term exposures (Appendix J).

Birds.

Toxicity studies have been conducted on the acute toxicity and reproductive effects of tebufenozide in birds, and a field study is available on reproductive effects. The acute toxicity of tebufenozide is low for birds (Appendix J).

Reproduction studies were conducted in mallard ducks (Beavers and others 1993a) and bobwhite quail (Beavers and others 1993b, Reinert 1995a). Dietary concentrations less than or equal to 1,000 ppm tebufenozide did not cause reproductive effects in mallard ducks. In the quail studies results are inconsistent. In a study by Beavers and others (1993b), reproductive effects included reduced number of eggs laid, viable embryos and 14-day-old survivors at dietary concentrations of 300 and 1,000 ppm, but not at 100 ppm. A similar study yielded no substantial dose-related effects in quail exposed to dietary concentrations of up to 615 ppm (Reinert 1995a).

A field study on the reproductive performance of Tennessee warblers (*Vermivora peregrina*) in forests treated with tebufenozide has been published (Holmes 1998). In this study, tebufenozide was applied twice at a rate of approximately 0.06 lb a.i./acre with a 4-day interval between applications, in a forest area in Ontario, Canada. Reproductive parameters assayed included number of eggs laid, percent hatch, and growth of the hatchlings as compared with an untreated control plot. A total of six nests were observed in the control plot, and five nests were treated with tebufenozide in the test plots, with no statistically significant adverse effects noted. However, there were decreases in both the average number of eggs per nest (6.3 in the control area and 5.8 in the treated area) as well as the percent hatch (97.4 percent in the control area and 89.7 percent in the treated area). The small sample sizes result in a low statistical power, and the results are "suggestive, although not necessarily compelling, that reproductive parameters were consistently lower in the treated blocks than in the control block" (Holmes 1998, p. 191). Some

differences in adult behavior were observed in the plot treated with tebufenozide, such as an increase in foraging time and an associated decrease in brooding time. This suggests that the primary effect on the birds may have been a decrease in food abundance.

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

Terrestrial Invertebrates.

While tebufenozide will be specifically used by the USDA Forest Service for the control of the gypsy moth, tebufenozide is effective in controlling other pest species, including the apple bud moth (*Platynota idaeusalis*) (Biddinger and others 1998), various species of spruce budworm (Payne and others 1997; Retnakaran and others 1997a, b), and the Indian-meal moth (*Plodia interpunctella*) (Oberlander and others 1998). A complete list of the pest species for which tebufenozide is specified is provided in U.S. EPA (1999e).

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

While 20-hydroxyecdysone is a hormone common to many invertebrates, the effectiveness of tebufenozide in mimicking 20-hydroxyecdysone activity seems to vary among orders and species of invertebrates. Although

the specificity of tebufenozide is not addressed in detail in the recent U.S. EPA (1999e) ecological risk assessment, it was reviewed in detail by Rohm and Haas (Keller 1998). That review is consistent with publications in the open literature relating to species specificity of tebufenozide (Addison 1996; Biddinger and Hull 1995; Biddinger and others 1998; Brown 1996; Butler and others 1997; Dhadialla and others 1998; Rumpf and others 1998; Smagghe and others 1996; Valentine and others 1996). In general, Lepidoptera are sensitive to tebufenozide, but other insects are much less sensitive (Smagghe and Degheele 1994a). The differing levels of sensitivity appear to be related to differences in ecdysone receptor binding (Smagghe and others 1996) rather than differences in pharmacokinetics (Smagghe and Degheele 1994b).

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

In the study by Addison (1996), tebufenozide was incorporated into forest soil at a concentration of 72.1 ppm. Based on a typical application rate of 70 g/ha and the assumption that tebufenozide will remain in the top 2 cm of soil, Addison (1996) estimated that the soil concentration of 72.1 ppm is equivalent to a concentration that is 100 times greater than expected environmental concentrations. There were no adverse effects on one species of earthworm (*Dendrobaena octaedra*) or on four species of Collembola (*Folsomia candida, Folsomia nivalis, Onychiurus parvicornis, and Hypogastrura pannosa*), which are indigenous to forest soils in Canada and the northern United States.

Consistent with results of the Addison (1996) study, a standard bioassay on earthworms (*Eisenia foetida*) noted no adverse effects at soil concentrations of up to 1,000 ppm over a 14-day exposure period (Garvey 1992).

Butler and others (1997b) conducted a study on canopy arthropods in which tebufenozide was applied at rates of 0.03 and 0.06 lb a.i./acre to a mixed oak plot in Ohio. The investigators examined the efficacy of tebufenozide against gypsy moth larvae and its effects on nontarget arthropods. Population assays included measures of abundance and diversity in 10 arthropod families and 15 lepidopteran species. A decrease in abundance was noted in some lepidopteran species, while no effects on abundance or richness were noted in any organisms other than lepidopteran species.

The studies by Valentine and others (1996) and Walgenbach (1995) involve the application of tebufenozide formulations to apple orchards. Tebufenozide had no effects on species of mites, spiders, various beetles (Coleoptera), and true bugs (Hemiptera), after being applied to apple orchards at rates effective in controlling lepidopteran pest species (Valentine and others 1996). Similarly, Walgenbach (1995) noted no effects on beneficial insect populations. These two studies support the general conclusion that tebufenozide is likely to have an adverse impact on Lepidoptera, but not on nonlepidopteran species.

Fish.

Information on the toxicity of tebufenozide to fish is summarized in Appendix J. All of the available studies were conducted in support of the registration of tebufenozide and submitted to the U.S. EPA.

The acute toxicity of tebufenozide to fish is relatively low with LC_{50} values of 3.0 mg a.i./L in bluegill sunfish (Graves and Smith 1992b) and 5.7 mg a.i./L in rainbow trout (Graves and Smith 1992c). There is greater concern, however, regarding the potential

chronic toxicity of tebufenozide to fish. The U.S. EPA evaluates all studies like those summarized in Appendix J to determine whether the conclusions are consistent with the data, and in many instances the U.S. EPA accepts the study conclusions. For tebufenozide, however, the U.S. EPA has disagreed with conclusions for a fathead minnow egg and fry study as well as a fathead minnow full life cycle study. This disagreement is discussed further in the dose-response assessment (section 4.3.3.1 of Appendix J).

Aquatic Invertebrates.

Unpublished studies on the toxicity of tebufenozide to aquatic invertebrates submitted to the U.S. EPA in support of the registration of tebufenozide are summarized in Appendix J. Some invertebrate assays were conducted in support of the registration of tebufenozide, and the summaries of these studies in Appendix J are based on a review of the full text copies of the studies submitted to the U.S. EPA. Additional studies published in the open literature are discussed below. Unlike some of the fish studies, the studies on aquatic invertebrates, summarized in Appendix J, were accepted without exception by the U.S. EPA (1999e).

In the studies submitted for registration, the acute toxicity of tebufenozide to *Daphnia* (Crustacea) and midges (Insecta) is on the same order as that for fish, with a 48-hour LC_{50} value of 3.8 mg/L for daphnids (Graves and Smith 1992a) and a 96-hour LC_{50} value of 0.3 mg/L for midge larvae (van der Kolk 1997). Similarly, a study published in the open literature and sponsored by the U.S. Geological Survey, reported higher LC_{50} values for Crustacea (17.37 mg/L for *Daphnia* and 5.53 mg/L for *Artemia*) than for two species of mosquitoes (0.92 mg/L for *Aedes aegypti* and 0.15 mg/L for *Aedes taeniorhynchus*) (Song and others 1997).

Kreutzweiser and Thomas (1995) assayed the effects of tebufenozide on aquatic invertebrate communities in lake enclosures. A dose-related decrease in cladoceran

abundance was noted, persisting for 1-2 months at the two lower concentrations and for 12-13 months at the two higher concentrations. The decrease in cladoceran abundance was accompanied by an increase in the abundance of rotifers, suggesting that the changes in community structure could be attributable to secondary or trophic effects rather than to toxicity.

Rohm and Haas summarized the results of several field studies or field simulation studies (Kreutzweiser and others 1994, 1995) regarding the effects of tebufenozide to aquatic invertebrates (Keller 1998).

Cumulative Effects of Tebufenozide.

Risk to Human Health (Issue 1).
Tebufenozide and diflubenzuron could have a cumulative effect on methemoglobinemia. USDA gypsy moth management programs do not use these two chemicals together in the same area at the same time; however, tebufenozide might be applied to the same area in multiple years for eradication projects. These multiple applications of tebufenozide over a period of time may increase the potential risk of methemoglobinemia. Exposure to other methemoglobinemia-inducing compounds in the environment may contribute to a cumulative effect. For example, individuals exposed to combustion smoke or carbon monoxide (agents causing oxidative damage to blood) in addition to exposure to tebufenozide may be at increased risk of developing methemoglobinemia. Individuals exposed to high levels of nitrates, either in air or in water, demonstrate increased levels of methemoglobin and may be at increased risk with exposure to compounds such as tebufenozide.

Risk to Nontarget Organisms (Issue 2).
Tebufenozide generally would not be used in conjunction with other treatments; however, it might be applied to the same area in multiple years for eradication projects. Generally these areas are small (usually no more than 5,000 acres). Tebufenozide might have a cumulative effect on nontarget caterpillars

of moths and butterflies by potentially reducing their populations, but it will not affect other aquatic and terrestrial species as used in USDA gypsy moth treatment projects.

4.10 Consequences of Adding a New Treatment Under Alternative 3.

At this time a prediction can not be made as to what new treatments might become available in the future for the gypsy moth. Given the protocol built into Alternative 3 (see Chapter 2), the effects and cumulative effects associated with any treatment(s) would pose no greater risk to human health and nontarget organisms than are disclosed in this draft SEIS for the currently approved treatments and for tebufenozide.

4.11 Summary of Effects Including Cumulative Effects.

Risk to Human Health (Issue 1).

General.
During a gypsy moth outbreak, people are exposed to large numbers of gypsy moths and experience skin and eye irritation and respiratory system effects, sometimes to the extent that they may seek medical treatment. Although both *B.t.k.* and Gypchek may also cause these effects, these irritations most likely will be less intense than irritations from a gypsy moth outbreak. No other human health effects are plausible for Gypchek; for disparlure, no human health risks could be identified, the only effect being the nuisance of male moths attracted to people working with traps that contain the female gypsy moth sex pheromone, disparlure.

No human health effects are likely from exposure to diflubenzuron and tebufenozide at application rates used in USDA gypsy moth projects. With very high exposures, increases in methemoglobin, an abnormal blood pigment that reduces the oxygen-carrying capacity of the blood, might be detectable for both insecticides. Should high application rates (0.12 lbs/acre in two applications 3 days apart) of tebufenozide be used, ingestion of tebufenozide becomes a concern (for example, on contaminated fruit; the upper range for the HQ of 1.5 is for long-term consumption of fruit; see *Table 4-2*). Applications at these high levels are not likely to occur for USDA projects.

The risk posed by dichlorvos is greatest for people who might tamper with traps and receive high levels of dermal exposure, or who might ingest the insecticide strip contained in the trap (*Table 4-2*). The upper range of the HQ of 380 depicts a child ingesting a dichlorvos strip; this scenario has never been encountered in USDA projects.

Cumulative Effects.

Repeated defoliation over successive years by gypsy moth caterpillars increases the potential exposure and subsequent skin, eye, and respiratory reactions. All of the treatments would reduce this risk over time. Diflubenzuron and tebufenozide both evoke the formation of methemoglobin; however, these treatments would not be utilized at the same time in the same area. Improper handling of dichlorvos poses a cumulative risk to workers, especially if ventilation is inadequate and proper handling procedures are not followed.

Risk to Nontarget Organisms (Issue 2).

General.

Other than effects on trees, current data and literature on the gypsy moth reveal only minor effects on other terrestrial and aquatic organisms; studies were in many cases of short duration and evaluated only a segment of the ecosystem or only a few species. There is a general lack of long-term, multi-year studies measuring over decades the impact of the gypsy moth on ecosystems and terrestrial and aquatic species and systems. This deficiency of extended studies may mask and underestimate the long-term impacts of gypsy moth on terrestrial and aquatic systems. Gypchek, mass trapping (dichlorvos), and disparlure have no long- or short-term effects on nontarget terrestrial species (all hazard quotients are less than 0.01, see *Table 4-2*). Gypchek and dichlorvos in USDA treatment projects do not affect aquatic nontarget organisms. The highest calculated disparlure hazard quotient in any aquatic organism is 0.37 (some small aquatic invertebrates). Under normal conditions of USDA gypsy moth management projects, disparlure is not expected to impact aquatic organisms.

B.t.k. applications impact certain spring-feeding butterflies and moths. Many lepidopteran species are not affected, especially those not present in the treated foliage and species arriving in treatment areas after the *B.t.k.* has disappeared from the foliage.

Compared with any of the other treatments, diflubenzuron affects a greater variety of terrestrial and aquatic nontarget species: moths and butterflies, grasshoppers, parasitic wasps, aquatic insects, bottom-dwelling crustaceans, and immature free-floating crustaceans (*Table 4-2*).

Tebufenozide affects only Lepidopterans, having no other expected significant effect on other terrestrial species or aquatic invertebrates (*Table 4-2*). There is no expectation that tebufenozide would be used at the highest application rates in USDA treatment projects; as a result the hazard quotient derived from a mammal eating contaminated fruit is likely to be lower than 1.5 (*Table 4-2*).

Cumulative Effects.

Repeated spraying with *B.t.k.*, diflubenzuron, or tebufenozide is likely to decrease lepidopteran species populations if the same areas are sprayed over 2 or more years. An expected result of cumulative impact on sensitive lepidopteran species from repeated annual spraying with any of these treatments is reasonable, as is the expectation that repeated annual spraying with diflubenzuron would have a cumulative impact on aquatic organisms if this insecticide reached aquatic ecosystems.

4.12 Operational Flexibility of Treatments.

For example, in order to minimize possible effects on threatened and endangered species that may be present in areas proposed for treatment, Gypchek, mass trapping, and mating disruption (where appropriate) could be selected instead of using *B.t.k.*, diflubenzuron, or tebufenozide.

Tebufenozide (Alternative 2) provides the USDA gypsy moth management program with an additional treatment option that may prove useful for reducing the threat posed by gypsy moth outbreaks. Alternative 3 affords the greatest flexibility to the USDA gypsy moth management program.

4.13 Unavoidable Adverse Effects.

Since this draft SEIS is programmatic in nature, no unavoidable adverse effects were identified for any of the alternatives. Any adverse effects that might occur would be identified and addressed in environmental analyses at the site-specific project level.

4.14 Short-Term Uses and Long-Term Productivity.

The National Environmental Policy Act (NEPA) requires consideration of "the relationship between short-term uses of man's environment and the mainte-nance and enhancement of long-term productivity" [42 U.S.C. 4322 (2)(C)]. As declared by the Congress, this relationship includes using all practicable means and measures, including financial and technical assistance, in a manner calculated to foster and promote the gen-eral welfare, to create and maintain conditions under which man and nature can exist in productive harmony, and fulfill the social, economic, and other requirements of present and future generations of Americans (NEPA Section 101).

The gypsy moth threatens the forest resources in the United States both in the short and long term, as described in Section 4.3 and in Appendix L. Each alternative provides treatments to lessen and delay the impacts of the gypsy moth on these forest resources. Alternative 2 provides an additional treatment and increased operational flexibility for gypsy moth treat-ment projects. Alternative 3 provides the greatest op-erational flexibility for gypsy moth treatment projects. Although the treatments may have short-term effects as outlined in Sections 4.4 – 4.9 and Table 4-2, no long-term effect could be identified--except for B.t.k. where sensitive spring lepidopteran species may take longer to recover. Mitigation measures at the site-specific proj-ect level will reduce the short- and long-term impacts of the treatments for each of the alternatives.

4.15 Measures to Mitigate Adverse Environmental Impacts.

Given the variety of places and circumstances where gypsy moth projects could be implemented, it will be necessary to develop and implement specific mitigation measures for each project. Mitigation measures will be developed and implemented on a site-specific basis for each project based on local conditions and concerns. See Chapter 2 for mitigation measures.

4.16 Urban Quality, Historic and Cultural Resources, and Design of the Built Environment.

In-depth, site-specific project environmental analyses will be performed for individual projects, as this draft SEIS is programmatic in nature.

4.17 Energy Requirements and Conservation Potential of Various Alternatives.

All of the alternatives involve energy use, primarily aviation fuel used by aircraft and helicopters for treatment application. Designing spray blocks for efficiency reduces flight time and conserves fuel.

4.18 Natural or Depleted Resource Requirements and Conservation Potential of Various Alternatives.

All alternatives reduce the impact of the gypsy moth on forest resources in protecting forests from gypsy moth outbreaks that may cause tree mortality. Other than the use of air space over treatment areas, with the short-term impacts of aviation noise and limitation of public use during application, no inherent natural or cultural

resource requirements exist for the three alternatives. Impacting factors for specific projects will be addressed with site-specific environmental analyses.

4.19 Irreversible and Irretrievable Commitments of Resources.

Irreversible and irretrievable commitments of resources due to the presence of the gypsy moth, defoliation, and specific treatments occur at the project level and are disclosed through site-specific analyses.

4.20 Other Required Disclosures.

NEPA at 40 CFR 1502.25(a) directs "to the fullest extent possible, agencies shall prepare draft environmental impact statements concurrently with and integrated with … other environmental review laws and executive orders."

Because this draft SEIS is programmatic in nature, the Forest Service and APHIS will ensure that site-specific consultations will be done as necessary at the project level for the Endangered Species Act (ESA), the National Historic Preservation Act (NHPA), and any other laws, regulations, executive orders, and agency policies that apply.

Chapter 5
Preparers and Contributors

Figure 5-1. Civilian Conservation Corps workers traveled by truck to perform gypsy moth field work.

Chapter 5 Preparers and Contributors

Contents

Figure

Individuals listed as preparers were responsible for developing the content of this document. Contributors shared information and expertise. Those named under Information Management assembled the document, posted material on the Web, and managed supporting information.

5.1 Preparers.

Interdisciplinary Team

Joseph L. Cook Supervisory Entomologist and SEIS Team Leader, USDA Forest Service, Northeastern Area State and Private Forestry, Newtown Square, PA.

Three years with the Forest Service and a total of 32 years of government service to various agencies and military branches, including the Navy, Army and Marine Corps. Positions held in the fields of natural resources, fisheries, marine biology, forestry, pest management, entomology, wildlife biology, cultural resources management, environmental management and planning. Participated in National Environmental Policy Act document preparation, implementation and administration at the local, regional and national level in a variety of assignments both in the United States and overseas. Served in the U.S. Army (active and reserve) as a Medical Entomologist. Academic degrees include a B.S. in Natural Resources from the University of Michigan in 1970, M.S. in Entomology (Forest Entomology) from the University of Minnesota in 1996 and M.B.A. from University of the Incarnate Word in 1991.

Weyman Fussell SEIS Team Co-Leader, USDA APHIS Plant Protection and Quarantine, Riverdale, MD.

Eight years with the USDA, including 3 years with APHIS Pest Detection and Management Programs

as Gypsy Moth program manager working to address phytosanitation issues domestically and internationally, focusing on Latin America. Prior to joining the USDA, taught at the university level for 5 years and spent 15 years in overseas programs addressing food production planning and implementation. Academic degrees include M.S. from Purdue University in Crop Genetics with a minor in Plant Pathology, and a Ph.D. from the University of Tennessee in 1983 in Agricultural Genetics with a minor in Economics of International Development.

Derek Handley Public Affairs Specialist, USDA Forest Service, Northeastern Area State and Private Forestry, Newtown Square, PA. (No longer with Forest Service)

Two years with the Forest Service, 11 years with the U.S. Navy and U.S. Navy Reserves. Responsibilities included community relations, speech writing, and media relations. Earned B.A. in English from Hampton University in 1994.

William Oldland Entomologist, USDA Forest Service, Northeastern Area State and Private Forestry, Newtown Square, PA.

Three years as an Entomologist with the USDA Forest Service and 8 years with the Tennessee Valley Authority (TVA) as contract and Federal employee. Served as TVA's Medical Entomologist for 2 of these 8 years; responsibilities included monitoring mosquito populations for West Nile Virus, malaria, several strains of encephalitis (EEE, SLE, LAC) and writing the vector chapters for the TVA EIS/Reservoir Operations Study. While serving as an Environmental Scientist for the TVA, his duties included sample collection, evaluation and compliance report composition for TVA Power Plants. While a contract Entomologist at the TVA, he assisted in the management of a biological control program for hydrilla and purple loosestrife. Bill

also spent 2 years in private industry as a Wildlife Biologist/Forester. He earned a B.S. in Wildlife Management in 1991 and M.S. in Entomology in 1993 from West Virginia University.

Mary Ann White Writer-Editor, USDA Forest Service, Northeastern Area State and Private Forestry, Newtown Square, PA. (No longer with Forest Service)

Two years with the Forest Service, 6 years on active duty in the U.S. Navy. Received A.S. in Medical Laboratory Technology from The George Washington University in 1979 and B.A (1984) and M.A.(1986) degrees in history from the University of Texas at El Paso.

Human Health and Ecological Risk Assessment Contractor

Syracuse Environmental Research Associates, Inc., Fayetteville, NY

5.2 Contributors.

Contributors provided information and expertise.

Management Group

USDA Forest Service advisory group on national gypsy moth policy.

Robert D. Mangold USDA Forest Service, Director, Forest Health Protection, Washington, DC

Jerry Boughton USDA Forest Service, Assistant Director Forest Health and Economics Programs, Northeastern Area State and Private Forestry, Newtown Square

Individuals

USDA Forest Service, USDA Animal and Plant Health Inspection Service (APHIS) and other USDA contacts provided assistance to the interdisciplinary team with their time, materials, critical review skills and support.

Debra Allen-Reid USDA Forest Service, Southwestern Region, Albuquerque, NM

John Anhold USDA Forest Service, Southwestern Region, Flagstaff, AZ

Hank Appleton USDA Forest Service, Forest Health Protection, Washington, DC

David Bakke USDA Forest Service, Pacific Southwest Region, Vallejo, CA

David A. Bergsten USDA APHIS, Riverdale, MD

Beverly M. Bulaon USDA Forest Service, Northern Region, Missoula, MT

David R. Bridgewater USDA Forest Service, Pacific Northwest Region, Portland, OR

Robert Cain USDA Forest Service, Rocky Mountain Region, Lakewood, CO

Joseph Carbone USDA Forest Service, Ecosystem Management Coordination, Washington, DC

William A. Carothers USDA Forest Service, Southern Region, Asheville, NC

Michael D. Connor USDA Forest Service, Northeastern Area State and Private Forestry, St. Paul, MN

Jesus A. Cota USDA Forest Service, Forest Health Protection, Washington, DC (retired)

Frank J. Cross USDA Forest Service, Rocky Mountain Region, Denver, CO (retired)

Meredith Dahl USDA, Office of the General Counsel, Washington, DC

John William Dale USDA Forest Service, Pacific Southwest Region, Vallejo, CA (retired)

Jack P. Edmundson USDA APHIS, Riverdale, MD

John H. Ghent USDA Forest Service, Southern Region, Asheville, NC

Kurt W. Gottschalk USDA Forest Service, Northern Research Station, Morgantown, WV

John W. Hazel USDA Forest Service, Northeastern Area State and Private Forestry, Morgantown, WV

Donna S. Leonard USDA Forest Service, Southern Region, Asheville, NC

Andrew M. Liebhold USDA Forest Service, Northern Research Station, Morgantown, WV

Jesse Logan USDA Forest Service, Rocky Mountain Research Station, Logan, UT

Leonard L. Lucero USDA Forest Service, Southwestern Region, Albuquerque, NM

Michael L. McManus USDA Forest Service, Northern Research Station, Hamden, CT

Victor C. Mastro USDA APHIS, Otis Plant Protection Center, Otis ANGB, MA

Paul A. Mistretta USDA Forest Service, Southern Region, Atlanta, GA

Wesley A. Nettleton USDA Forest Service, Southern Region, Atlanta, GA

Amy H. Onken USDA Forest Service, Northeastern Area State and Private Forestry, Morgantown, WV

Doug Parker USDA Forest Service, Southwestern Region, Albuquerque, NM (no longer with Forest Service)

John D. Podgwaite USDA Forest Service, Northern Research Station, Hamden, CT

Bernard J. Raimo USDA Forest Service, Northeastern Area State and Private Forestry, Durham, NH

Richard C. Reardon USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV

Leslie Rubin USDA APHIS, Riverdale, MD

Dwight Scarbrough USDA Forest Service, Intermountain Region, Boise, ID

Noel F. Schneeberger USDA Forest Service, Northeastern Area State and Private Forestry, Newtown Square, PA

Dave E. Schultz USDA Forest Service, Pacific Southwest Region, Redding, CA (deceased)

Mark E. Schultz USDA Forest Service, Alaska Region, Southeast Alaska Field Office, Juneau, AK

David Sire USDA Forest Service, Ecosystem Management Coordination, Washington, DC

James M. Slavicek USDA Forest Service, Northern Research Station, Delaware, OH

Rhonda R. Solomon USDA APHIS, Riverdale, MD

Dennis J. Souto USDA Forest Service, Northeastern Area State and Private Forestry, Durham, NH

Harold Thistle USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV

Kevin Thorpe USDA Agricultural Research Service, Beltsville, MD

Patrick C. Tobin USDA Forest Service, Northern Research Station, Morgantown, WV

Kathryn Toffenetti USDA, Office of the General Counsel, Washington, DC

Daniel B. Twardus USDA Forest Service, Northeastern Area State and Private Forestry, Morgantown, WV

Algimantas P. Valaitis USDA Forest Service, Northern Research Station, Delaware, OH

Ralph Webb USDA, Agricultural Research Service, Beltsville, MD

5.3 Information Management and Analysis Staff.

The Information Management and Analysis Staff of the USDA Forest Service, Northeastern Area State and Private Forestry, provided valuable assistance in preparation and printing of the draft and final reports, Internet site management, and computer operations support.

Cindy Barnett Morgantown, WV

Roberta Burzynski Newtown Square, PA

Helen Butalla Morgantown, WV

Patty Dougherty Newtown Square, PA

Victoria Evans Morgantown, WV

Sandy Fosbroke Morgantown, WV

Nancy Lough Morgantown, WV

Juliette Watts Newtown Square, PA

Chapter 6
Mailing List

Figure 6-1. Early aerial gypsy moth treatments were manually released.

Chapter 6   Mailing List

Contents

Figure

This chapter lists agencies, organizations, libraries, and individuals who were mailed complete copies of the draft supplemental environmental impact statement or notified of its availability on the Web.

6.1 Federal Agencies

Alabama

United States Department of Agriculture (USDA), Forest Service, National Forests in Alabama (National Forests & Ranger Districts)

USDA, Animal Plant Health & Inspection Service (APHIS), Plant Protection & Quarantine (PPQ)

Alaska

United States Department of Interior (USDI), Bureau of Indian Affairs (BIA) Field Offices

National Marine Fisheries Service

USDA, APHIS, PPQ

USDA, Forest Service, Alaska Region

USDA, Forest Service, National Forests in Alaska (National Forests & Ranger Districts)

Arizona

USDA, APHIS, PPQ

USDA, Forest Service, National Forests in Arizona (National Forests & Ranger Districts)

USDI, BIA Field Offices

Arkansas

USDA, APHIS, PPQ

USDA, Forest Service, National Forests in Arkansas (National Forests & Ranger Districts)

California

USDI, BIA Field Offices

USDI, Bureau of Land Management (BLM)

United States Environmental Protection Agency (USEPA), Region IX

Federal Aviation Administration, Western Region

National Marine Fisheries Service

U.S. Army Corps of Engineers, South Pacific Division (CESPD)

USDA, APHIS, PPQ

USDA, Forest Service, Region

USDA, Forest Service, National Forests in California (National Forests & Ranger Districts)

Colorado

USDI, BIA, Field Offices

United States Environmental Protection Agency (USEPA), Region VIII

USDA, Forest Service, Comanche National Grassland

USDI, National Park Service (NPS), Intermountain Regional Office

USDA, Forest Service, Pawnee National Grassland

USDA, APHIS, PPQ

USDA, Forest Service, National Forests in Colorado (National Forests & Ranger Districts)

USDA, Forest Service, Rocky Mountain Region

Connecticut

USDA, Forest Service, National Forests in Connecticut (National Forests & Ranger Districts)

USDA, Forest Service, Northern Research Station

Delaware

USDI, U.S. Fish and Wildlife Service (USFWS), Bombay Hook National Wildlife Refuge (NWR)

USDA, APHIS, PPQ

District of Columbia

Advisory Council on Historic Preservation

Department of Defense (DOD), Armed Forces Pest Management Board

DOD, U.S. Navy, Office of Chief of Navy Operations

Department of Energy (DOE), Office of Environmental Compliance Rural Energy Regulatory Commission, Environmental Compliance Branch

Department of Housing & Urban Development (HUD), Office of Environment & Energy

DOI, USFWS, Division of Endangered Species

USEPA, Office of Environmental Affairs

USEPA, Office of Federal Activities

General Services Administration, Office of Planning & Analysis (OPA)

Rural Electrification Administration, Environmental Compliance

Susquehanna River Basin Commission

U.S. Army Corps of Engineers

U.S. Coast Guard, Environmental Impact Branch

U.S. Department of the Interior, Office of Environmental Affairs

U.S. Department of Transportation, Environmental Division

USDA OPA Publication Stockroom

USDA, Forest Service

USDA, Forest Service, Office of Environmental Coordination

Florida

USDI, BIA, Seminole Agency

National Marine Fisheries Service

USDA, APHIS, PPQ

USDA, Forest Service, National Forests in Florida (National Forests & Ranger Districts)

Georgia

USEPA, Region IV

U.S. Army Corps of Engineers, South Atlantic Division (CESAD)

USDA, APHIS, PPQ

USDA, Forest Service, National Forests in Georgia (National Forests & Ranger Districts)

USDA, Forest Service, Southern Region

Hawaii

U.S. Army Corps of Engineers, Pacific Ocean Division (CEPOD)

USDA, APHIS, PPQ

Idaho

USDA, APHIS, PPQ

USDI, NPS, Craters of the Moon National Monument

USDA, Forest Service, National Forests in Idaho (National Forests & Ranger Districts)

Illinois

USDI, USFWS, Crab Orchard NWR

USDI, USFWS, Cypress Creek NWR

USEPA, Region V

Federal Aviation Administration, Great Lakes Region

USDI, NPS, Lincoln Home National Historical Site

USDI, USFWS, Mark Twain NWR

U.S. Army Corps of Engineers, North Central Division (CENCD)

USDA, Forest Service, National Forests in Illinois (National Forests &Ranger Districts)

USDA, APHIS, PPQ

Indiana

USDA, APHIS, PPQ

USDA, Forest Service, National Forests in Indiana (National Forests & Ranger Districts)

Iowa

USDA, APHIS, PPQ

USDI, NPS, Effigy Mounds National Monument

USDI, NPS, Herbert Hoover National Historic Site

USDI, USFWS, Union Slough NWR

USDA, Forest Service, National Forests in Iowa (National Forests & Ranger Districts)

Kansas

USDA, Forest Service, Cimarron National Grasslands
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Kansas
(National Forests & Ranger Districts)

Kentucky

Ohio River Basins Commission
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Kentucky
(National Forests & Ranger Districts)

Louisiana

USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Louisiana
(National Forests & Ranger Districts)

Maine

USDI, NPS, Acadia National Park
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Maine
(National Forests & Ranger Districts)

Maryland

USDI, NPS, Antietam National Battlefield
USDI, NPS, Blackwater National Wildlife Refuge
USDI, USFWS, Eastern Neck NWR
USDI, USFWS, Patuxent Wildlife Resource Center
USDA, Agriculture Research Service (ARS), Insect
Biocontrol Laboratory
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Maryland
(National Forests & Ranger Districts)
USDA, National Agricultural Library

Massachusetts

USEPA, Region I
Federal Aviation Administration, New England Region
National Marine Fisheries Service
U.S. Army Corps of Engineers, New England Division
(CENED)

USDA, APHIS, PPQ
USDA, Forest Service, National Forests in
Massachusetts (National Forests & Ranger
Districts)

Michigan

USDI, BIA Field Offices
USDI, NPS, Isle Royal National Park
USDI, USFWS, Shiawassee NWR
USDI, NPS, Sleeping Bear Dunes National Lakeshore
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Michigan
(National Forests & Ranger Districts)

Minnesota

USDI, USFWS, Agassiz NWR
USDI, BIA Field Offices
USDI, NPS, Grand Portage National Monument
USDI, USFWS, Pipestone National Monument
USDI, USFWS, Rice Lake NWR
USDI, USFWS, Tamarac NWR
USDI, USFWS, Upper Mississippi River NWR
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Minnesota
(National Forests & Ranger Districts)
USDI, NPS, Voyagers National Park

Mississippi

USDI, BIA Field Offices
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Mississippi
(National Forests & Ranger Districts)

Missouri

USDI, USFWS, Clarence Cannon NWR
USEPA, Region VII
Federal Aviation Administration, Central Region
USDI, NPS, Harry S. Truman National Historic Site
USDI, NPS, Ozark National Scenic Riverways
USDI, USFWS, Squaw Creek NWR
USDI, USFWS, Swan Lake NWR
USDA, APHIS, PPQ

USDA, Forest Service, National Forests in Missouri
    (National Forests & Ranger Districts)

Montana

USDI, BIA Field Offices
USDI, BLM
USDI, USFWS, Bowdoin NWR
USDI, NPS, Glacier National Park
USDI, NPS, National Bison Range
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Montana
    (National Forests & Ranger Districts)

Nebraska

USDI, BIA Field Offices
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Nebraska
    (National Forests & Ranger Districts)

Nevada

USDI, BIA Field Offices
USDI, BLM
USDI, NPS, Great Basin National Park
USDI, NPS, Spring Mountains National Recreational
    Area
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Nevada
    (National Forests & Ranger Districts)

New Hampshire

USDI, NPS, St. Glaudens National Historic Park
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in New
    Hampshire (National Forests & Ranger Districts)

New Jersey

Delaware River Basins Commission
USDI, NPS, Delaware Water Gap National Recreation
    Area
USDI, USFWS, Great Swamp NWR
USDI, NPS, Morristown National Historic Site

USDI, NPS, Shady Hook Gateway National Recreation
    Area
USDA, APHIS, PPQ

New Mexico

USDI, BIA Field Offices
USDI, NPS, National Park Service Intermountain
    Support Office
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in New
    Mexico (National Forests & Ranger Districts)

New York

USDI, BIA, Field Offices
USEPA, Region II
Federal Aviation Administration, Eastern Region
Federal Highway Administration, Region I
USDI, NPS, Fire Island National Seashore
USDI, USFWS, Montezuma National NWR
USDI, USFWS, Morton National Wildlife Refuge
USDI, NPS, Roosevelt-Vanderbilt National Historic
    Site
U.S. Army Corps of Engineers, North Atlantic
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in New York
    (National Forests & Ranger Districts)

North Carolina

USDA, APHIS, PPQ
USDA, Forest Service, National Forests in NC
  (National Forests & Ranger Districts)

North Dakota

USDI, BIA Field Offices
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in North
    Dakota (National Forests & Ranger Districts)

Ohio

USDI, USFWS, Cedar Point NWR
USDI, USFWS, Ottawa NWR

U.S. Army Corps of Engineers, Ohio River Division
(CEORD)
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Ohio
(National Forests & Ranger Districts)

Oklahoma

USDI, BIA, Field Offices
USDA, Forest Service, Black Kettle National
Grasslands
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Oklahoma
(National Forests & Ranger Districts)

Oregon

USDI, BIA Field Offices
USDI, BLM
USDI, NPS, Columbia Gorge National Scenic Area
USDI, NPS, Columbia River National Scenic Area
USDI, NPS, Hells Canyon National Recreation Area
Northwest Power Planning Council
USDI, NPS, Oregon Dunes National Recreation Area
U.S. Army Corps of Engineers, North Pacific Division
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Oregon
(National Forests & Ranger Districts)

Pennsylvania

USDI, NPS, Delaware Gap National Recreation Area
USEPA, Region III
USDI, USFWS, Erie NWR
USDI, USFWS, Lamar NWR
USDA Forest Service Northeastern Research Station
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in
Pennsylvania (National Forests & Ranger Districts)
USDI, NPS, Valley Forge National Historical Site

Rhode Island

USDA, APHIS, PPQ

South Carolina

Rural Development Administration, Region III-
Southeast
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in South
Carolina (National Forests & Ranger Districts)

South Dakota

USDI, BIA Field Offices
USDA, Forest Service, Fall River Rd/Wall Road/
Buffalo Gap National Grasslands
USDA, Forest Service, Fort Pierre National Grassland
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in South
Dakota (National Forests & Ranger Districts)

Tennessee

USDI, NPS, Great Smokey Mountains National Park
Tennessee Valley Authority (TVA), Environmental
Quality
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Tennessee
(National Forests & Ranger Districts)

Texas

USDA, BIA, Field Offices
USDA, Forest Service, Caddo LBJ National Grasslands
USEPA, Region VI
Federal Aviation Administration, Southwest Region
U.S. Army Corps of Engineers, Southwestern Division
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Texas
(National Forests & Ranger Districts)

Utah

USDI, NPS, Arches National Park
USDI, BIA Field Offices
USDI, BLM
USDI, NPS, Bryce Canyon National Park
USDI, NPS, Canyonlands National Park
USDI, NPS, Capital Reef National Park
USDI, NPS, Cedar Breaks National Monument
USDI, NPS, Flaming Gorge National Recreation Area

USDI, NPS, Natural Bridges National Monument
USDI, NPS, Timpanogos Cave National Monument
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Utah
(National Forests & Ranger Districts)
USDI, NPS, Zion National Park

Vermont

USDA, APHIS, PPQ

Virginia

USDI, NPS, George Washington Memorial Pkwy
USDI, NPS, Mount Rogers National Recreation Area
USDI, NPS, Shenandoah National Park
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Virginia
(National Forests & Ranger Districts)

Washington

USDI, BIA Field Offices
USEPA, Region X
Federal Aviation Administration, Northwest Region
USDI, NPS, Mt. Rainier National Park
USDI, NPS, North Cascades National Park
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Washington
(National Forests & Ranger Districts)

West Virginia

DoD-Army Blueston Lake, US Army Corps Of
Engineers
USDI, NPS, Harpers Ferry National History Park
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in West
Virginia (National Forests & Ranger Districts)

Wisconsin

USDI, NPS, Apostle Islands National Lakeshore
USDI, BIA, Field Offices
USDI, USFWS, Necedah NWR
USDI, NPS, St. Croix National Scenic Riverway
USDI, USFWS, Trempealeah NWR

USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Wisconsin
(National Forests & Ranger Districts)

Wyoming

USDI, BIA Field Offices
USDI, BLM
USDI, NPS, Grand Teton National Park
USDA, APHIS, PPQ
USDA, Forest Service, National Forests in Wyoming
(National Forests & Ranger Districts)
USDI, NPS, Yellowstone National Park

Puerto Rico

USDA, Forest Service, National Forests in Puerto Rico
(National Forests & Ranger Districts)

6.2 State and Local Agencies

Alabama

Alabama A&M University, Forestry Sciences
Laboratory
Cooperative Extension Service
Department of Agriculture & Industries
Forestry Commission

Alaska

Cooperative Extension Service
Department of Environmental Conservation
Department of Fish & Game
Department of Natural Resources
Division of Forestry
Project Analyst- Forests Alaska State Agencies

Arizona

Department of Agriculture
Department of Agriculture Plant Services Division
Game & Fish Department
State Land Department
State Parks

Arkansas

Cooperative Extension Service
Forestry Commission
State Plant Board
State Plant Pest Board Division of Plant Industry

California

Department of Environmental Protection
Department of Fish & Game
Department of Food & Agriculture
Department of Forestry & Fire Protection
Department of Parks & Recreation
Department of Water Resources
Redwood Sciences Laboratory
State Lands Commission

Colorado

Department of Agriculture
Department of Natural Resources
State Conservation Board
State Forest Service

Connecticut

Bureau of Natural Resources
Department of Environmental Protection
Division of Forestry
Middlesex County Cooperative Extension Service
State Forest Tree Nursery
University of Connecticut Cooperative Extension
    Service
University of Connecticut Storrs Agriculture
    Experiment Station
West Hartford Cooperative Extension Service
Windham County Cooperative Extension Service

Delaware

Delaware Department of Agriculture
Department of Natural Resources & Environment
Division of Fish & Wildlife
Forest Service
Kent County Cooperative Extension Service
New Castle County Cooperative Extension Service

Sussex County Cooperative Extension Service
University of Delaware CES

District of Columbia

DC Government

Florida

Cooperative Extension Service (CES)
Department of Agriculture & Consumer Services
    Division of Plant Industry
Department of Environmental Protection
Division of Forestry
Fish & Wildlife Conservation Commission
Pike County CES

Georgia

Department of Agriculture Plant Protection Division
Department of Natural Resources
Environmental Council
Environmental Policy Institute
Forestry Commission

Hawaii

Department of Agriculture Plant Industry Division
Department of Land & Natural Resources
Division of Forestry & Wildlife

Idaho

Department of Environmental Quality
Department of Fish & Game
Department of Lands Northern Operations
Department of Lands Southern Operations
Department of Parks & Recreation
Department of Agriculture Division of Plant Industry
Department of Lands
Idaho County Weed Supervisor
Northwest Watershed Research Center
Water Resources Department

Illinois

Adams County CES
Boone County CES

Brown County CES

Bureau County CES

Caroll County CES

Champaign CES

Charleston CES

Chicago Park District

Christian County CES

Clark County CES

Clay County CES

Clinton County CES

Cook County CES

Crawford County CES

Cumberland County CES

Dekalb County CES

Department of Agriculture

Department of Conservation

Department of Natural Resources

Department of Public Health

Department of Conservation Forbes State Fish &
     Wildlife Area

Division of Forest Resources

Douglas County CES

Dupage County CES

Edgar County CES

Edwards County CES

Effingham County CES

Environmental Council

Fayette County CES

Ford County CES

Forest Research Center

Franklin County CES

Fulton County CES

Gallatin County CES

Greene County CES

Grundy County CES

Hamilton County CES

Hancock County CES

Hardin County CES

Henderson County CES

Henry County CES

Iroquois County CES

Jasper County CES

Jefferson County CES

Jerseyville CES

Jo Daviess County CES

Johnson/Massac Counties CES

Jones County CES

Kane County CES

Kankakee County CES

Kendall County CES

Knox County CES

Lasalle County CES

Lawrence County CES

Lee County CES

Livingston County CES

Logan County CES

Macon County CES

Macon County, Soil & Water

Macoupin County CES

Madison County CES

Marion County CES

Marion Extension Center

Marshall/Putnam Counties CES

Mason County CES

Mcdonough County CES

Mchenry County CES

Mclean County CES

Menard County CES

Mercer County CES

Monroe County CES

Montgomery County CES

Morgan County CES

Moultrie County CES

Natural History Survey

Nature Preserves Commission

Ogle County CES

Oquawka CES

Peoria County CES

Peorial County CES

Perry County CES

Pittsfield CES

Pope/Hardin Counties CES

Randolph County CES

Region 1 CES

Region 2 CES

Region 4 CES

Region 6 CES
Region 7 CES
Richland County CES
Rock Island County CES
Saline County CES
Sangamon County CES
Scott County CES
Shelby County CES
St. Clair County CES
Stephenson County CES
Tazewell County CES
Union County CES
University of Illinois CES
Urbana CES
Urbana, Department of Forestry
Vermilion County CES
Wabash County CES
Warren County CES
Washington County CES
Wayne County CES
White County CES
Whiteside County CES
Will County CES
Williamson County CES
Winnebago Count CES
Woodford County CES

Indiana

Adams County CES
Allen County CES
Bartholomew County CES
Benton County CES
Boone County CES
Brown County CES
Carroll County CES
Cass County CES
Clark County CES
Clay County CES
Crawford County CES
Davless County CES
Dearborne County CES
Decator County CES
Delaware County CES

Department of Conservation
Department of Natural Resources
Department of Health
Division of Entomology & Plant Pathology
Division of Fish & Wildlife
Division of Forestry
Division of Forestry Department of Natural Resources
Dubois County CES
Elkhart County CES
Fayette County CES
Floyd County CES
Fountain County CES
Franklin County CES
Fulton County CES
Gibson County CES
Grant County CES
Green County CES
Hamilton County CES
Hancock County CES
Harrison County CES
Hendricks County CES
Henry County CES
Howard County CES
Huntington County CES
Jackson County CES
Jasper County CES
Jay County CES
Jefferson County CES
Jennings County CES
Johnson County CES
Knox County CES
Kosciusko County CES
Lagrange County CES
Lake County CES
Laporte County CES
Lawrence County CES
Madison County CES
Marion County CES
Marshall County CES
Martin County CES
Miami County CES
Monroe County CES
Montgomery County CES

Morgan County CES
Newton County CES
Noble County CES
Owen County CES
Parke County CES
Perry County CES
Porter County CES
Posey County CES
Pulaski County CES
Purdue University, State Extension Forester
Putnam County CES
Randolph County CES
Ripley County CES
Rush County CES
Scott County CES
Shelby County CES
Starke County CES
Steuben County CES
Sullivan County CES
Switzerland County CES
Tippecano County CES
Tipton County CES
Union County CES
Vanderburgh County CES
Vermillion County CES
Vigo County CES
Wabash County CES
Warren County CES
Warrick County CES
Washington County CES
Wayne County CES
Wells County CES
White County CES
Whitley County CES

Iowa

Adair County CES
Adams County CES
Allamake County CES
Audubon County CES
Bloomfield County CES
Boone County CES
Bremer County CES

Buchanan County CES
Buena Vista County Vista CES
Butler County CES
Calhoun County CES
Carroll County CES
Cedar County CES
Cerro Gordo County CES
Cherokee County CES
Chickasa County CES
Clark County CES
Clay County CES
Clayton County CES
Clinton County CES
Council Bluffs CES
Dallas CES
Decatur CES
Delaware CES
Department of Agriculture State Horticulturist
Department of Forestry
Department of Natural Resources
Department of Natural Resources State Forest Nursery
Department of Agriculture
Des Moines County CES
Dubuque County CES
Emmet County CES
Fayette County CES
Floyd County CES
Franklin County CES
Freemont County CES
Greene County CES
Grundy County CES
Guthrie County CES
Hamilton County CES
Hamilton County Conservation Board
Hancock CES
Hardin County CES
Harrison County CES
Henry County CES
Howard County CES
Humboldt County CES
Ida Grove County CES
Iowa County CES
Iowa Department of Agriculture

Iowa Department of Public Health
Iowa State University CES
Jackson County CES
Jasper County CES
Jones County CES
Keokuk County CES
Kossuth County CES
Lee County CES
Loess Hills State Park
Louisa County CES
Lucas County CES
Lyon County CES
Madison County CES
Mahaska County CES
Marion County CES
Marshall County CES
McFarland Park
Melvern CES
Mitchell County CES
Monona County CES
Monroe County CES
Montgomery County CES
Muscatin County CES
Oakland CES
O'Brien County CES
Osceola County CES
Palo Alto County CES
Plymouth County CES
Pocahontas County CES
Polk County CES
Ringold County CES
Sac County CES
Scott CES
Shimek State Forest
Sigourney CES
Sioux CES
Spirit Lake CES
State Entomologist
State Forest Nursery
Stephens State Forest
Story County CES
Taylor County CES
Toledo CES

Union County CES
Urbana CES
Van Buren County CES
Vinton CES
Wapello County CES
Washington County CES
Wayne County CES
Webster County CES
Winnebago County CES
Winneshi CES
Worth County CES
Wright County CES
Yellow River State Forest

Kansas

Department of Agriculture Plant Prot. & Weed Control Program
Department of Wildlife & Parks
Forest Service

Kentucky

CES
Department of Fish & Wildlife Resources
Director, Kentucky Division of Forestry
Natural Resources & Environmental
Office of State Entomologist

Louisiana

Agcenter, Louisiana CES
Department of Agriculture & Forestry
Department of Natural Resources
Department of Wildlife & Fisheries

Maine

Department of Agriculture
Division of Plant Industry, Department of Agriculture
Forest Service
University of Maine CES

Maryland

Allegany County CES
Ann Arundel CES

Baltimore County CES
Calvert County CES
Caroline County CES
Carroll County CES
Charles County CES
Department of Agriculture
Department of Agriculture Forest Pest Management
Department of Agriculture, Entomology Nursery
    Inspection
Department of Natural Resources, Forest Service
Dorchester County CES
Garrett County CES
Gypsy Moth Program, Prince Georges County
Hartford County CES
Howard County CES
Kent County CES
Montgomery County Gypsy Moth Program
Prince Georges County CES
Somerset County CES
Talbot County CES
Wicomico County CES
Worcester County CES

Massachusetts

Association of Conservation Commissions
Barnstable County CES
Berrien CES
Central Massachusetts Extension Center
Pittsfield CES
Department of Conservation
Department of Environmental Management
Department of Environmental Management Region 5
Headquarters
Department of Food & Agriculture
Division of Fisheries & Wildlife
Division of Forests & Parks
Division of Regulatory Services, Department of
Agriculture Resources
Division State Parks & Recreation
East Massachusetts Extension Center
Middlesex County CES
Pioneer Valley Extension Center

Plymouth County CES
Southeast Massachusetts Agriculture Center

Michigan

Alabastor Township
Alger & Marquette Soil Conservation District
Alger County CES
Allendale Charter Townships
Alpena County CES
Alpena Township
Antrim County CES
Antrim County Gypsy Moth Program
Arenac County CES
Arenac County Gypsy Moth Coordinator
Barry County CES
Bay County CES
Bay County Gypsy Moth Program
Bay County Gypsy Moth Suppression Program
Benzie CES
Bloomfield Township
Branch CES
Calhoun CES
Camden Township
Cass County CES
Charlevois CES
Charter Township of Highland
Charter Township of West Bloomfield
Cheboygan CES
Cherry Grove Township
Chippewa CES
Chippewa County CES
Clare County CES
Clare County Gypsy Moth Program
Crawford County CES
Crawford County Gypsy Moth Coordinator
Delta County CES
Department of Agriculture
Department of Agriculture Pesticide & Plant Pest
Management Division
Department of Natural Resources
Department of Natural Resources Forestry Division
Department of Public Health
Dickson Township Manistee County

Eaton City CES
Emmet County CES
Fenton Township
Frankenmuth Township
Genesee County Gypsy Moth Coordinator
Genessee County CES
Gladwin City CES
Gladwin County Gypsy Moth Coordinator
Gogebic CES
Grand Traverse County CES
Gration County Gypsy Moth Coordinator
Harrisville CES
Hawes Township Trustee
Hillsdale CES
Home Township
Houghton CES
Huron City CES
Iosco County CES
Iosco County Gypsy Moth Coordinator
Iron County CES
Iron Mountain CES
Isabella County
Jackson CES
Kalamazo CES
Kalkaska County Gypsy Moth Coordinator
Kalkaska County Gypsy Moth Program
Kent County CES
Kent County Conservation District
Lake CES
Lake County Commissioners
Lake County Gypsy Moth Program
Lansing CES
Leelanau County CES
Lenawee County CES
Livingston County Gypsy Moth Coordinator
Livingston County Gypsy Moth Program
Luce County CES
Mackinac County CES
Macob City CES
Manistee CES
Manistee County
Manistee County Commission
Manistee County Planning Commission

Manistee Soil & Water Conservation District
Marion Township
Marquette CES
Mason County CES
Mecosta County Gypsy Moth Coordinator
Menomine CES
Michigan Department of Agriculture
Michigan Department of Transportation
Michigan State University Extension
Midland County CES
Midland County Gypsy Moth Program
Midland County Gypsy Moth Suppression Program
Millen Township Supervisor
Mio CES
Missaukee County Gypsy Moth Coordinator
Missaukee County CES
Monroe County CES
Montcalm County CES
Montgomery CES
Newaygo CES
Newaygo County Gypsy Moth Coordinator
Newaygo County Gypsy Moth Suppression Program
Oakland County CES
Oakland County Gypsy Moth Program
Oceana CES
Ogemaw County Gypsy Moth Coordinator
Ontonago CES
Osceola County Gypsy Moth Program
Oscoda County Gypsy Moth Coordinator
Otsego County CES
Ottawa County Gypsy Moth Coordinator
Ottawa County Gypsy Moth Program
Pesticide & Plant Pest Management Division,
Department of Agriculture
Presque Isle CES
Region 2, Michigan Department of Natural Resources
Rochester Parks Department
Roscommon County
Roscommon County Gypsy Moth Suppression Program
Saginaw County CES
Saginaw County Gypsy Moth Coordinator
Sanilac County CES
Shiawassee County Gypsy Moth Coordinator

St. Clair County CES
St. Jose CES
Sweetwater Township
Tuscola County CES
Van Buren CES
Village of Beverly Hills
Wexford CES
Wexford County Gypsy Moth Coordinator

Minnesota

Aitkin County CES
Becker County CES
Beltrami County CES
Benton County CES
Big Stone County CES
Blue Earth County CES
Brown County CES
Brown County Historical Society
Cass County CES
Chippewa County CES
Chisago County CES
Clay County CES
Clearwater County CES
Clearwater County Land Department
Clearwater County Land Forestry Department
Cook County CES
Cottonwood County CES
Crow Wing County CES
Dakota County CES
Department of Agriculture
Department of Agriculture Agronomy & Plant Protection
Department of Natural Resources
Department of Natural Resources
Department of Public Health
Division of Forestry
Dodge County CES
Douglas County CES
Faribault County CES
Fergus Falls CES
Goodhue City County CES
Graceville Township
Grant County CES

Hennepin County CES
Houston County CES
Hubbard County CES
Huntsville Township
Isanti County CES
Itasca County Extension
Jackson County CES
Kanabec County CES
Kandiyohi County CES
Kittson County CES
Koochiching CES
Lac Qui Parle CES
Lake County CES
Lake of the Woods CES
Le Seur County CES
Lincoln County CES
Lyon County CES
Mahnomen County CES
Marshall County CES
Martincounty CES
McLoud County CES
Mille Lacs County CES
Morrison County CES
Mower County CES
Murray County CES
Nicollet County CES
Nobles County CES
Norman County CES
Olmsted County CES
Ore County CES
Owatonna CES
Pennington County CES
Perham CES
Pine County CES
Pipestone CES
Polk West County CES
Ramsey County CES
Red Lake City CES
Redwood County CES
Renville County CES
Rice County CES
Rock County CES
Roseau County CES

St. Louis County Land Department
Sibley County CES
St. Louis CES
Stearns County CES
Stearns County Park Department
Stevens County CES
Swift County CES
Todd County CES
Traverse County CES
University of Minnesota CES
Wabasha County CES
Wadena County CES
Waseca County CES
Washington County CES
Watonwan County CES
Wilkin County CES
Winona County CES
Wright County CES
Yellow Medicine CES

Mississippi

Department of Wildlife, Fisheries
Division of Plant Industry
Forestry Commission

Missouri

Adair County CES
Andrew County CES
Atchison City CES
Audrain County CES
Audrain County CES
Barry County CES
Barton County CES
Bates County CES
Benton County CES
Boone County CES
Buchanan City CES
Butler County CES
Caldwell County CES
Callaway County CES
Camden City CES
Carroll City CES

Carter County CES
Cass County CES
Cedar County CES
Chariton County CES
Christian City CES
Clark County CES
Clinton County CES
Cole County CES
Cooper County CES
Crawford County CES
Dade County CES
Dallas County CES
Daviess County CES
Dekalb County CES
Delta Center County CES
Dent County CES
Department of Agriculture
Department of Conservation
Department of Health
Department of Natural Resources
Douglas County CES
Dunklin County CES
Gasconade County CES
Green County CES
Grundy City CES
Harrison County CES
Hickory City CES
Hickory County CES
Hold County CES
Howard County CES
Howell County CES
Jackson County CES
Jasper County CES
Knox County CES
Laclede City CES
Laclede County CES
Lafayette City CES
Lawrence County CES
Lebanon Excess Property Center
Lewis County CES
Lincoln County CES
Linn County CES
Livingston County CES

Macon County CES
Madison County CES
Maries County CES
Marion County CES
Marles County CES
Mercer County CES
Miller City CES
Mississippi County CES
Missouri CES
Moniteau City CES
Monroe County CES
Montgomery County CES
Morgan County CES
Natural History Program
Newton County CES
Nordaway City CES
Oregon City CES
Oregon County CES
Osage City CES
Ozark County CES
Pemiscot City CES
Pettis County CES
Phelps City CES
Phelps County CES
Pike County CES
Plant Industries Division
Platte County CES
Polk County CES
Pulaski CountyCES
Putnam County CES
Randolph County CES
Ralls City CES
Ray County CES
Ripley County CES
Saline County CES
Schuyler County CES
Scotland County CES
Scott County CES
Shelby City CES
St Loius CES
St. Charles County CES
St. Clair County CES
St. Louis City CES

Ste. Genevieve City CES
Stoddard County CES
Sullivan County CES
Taney County CES
Texas County CES
University of Missouri CES
Vernon County CES
Warren County CES
Washington County CES
Webster City CES
Webster County CES
Worth County CES
Wright County CES

Montana

Department of Agriculture
Department of Natural Resources & Conservation
Department of Fish & Wildlife
Forestry Division

Nebraska

Department of Agriculture Bureau of Plant Industry
Department of Environmental Quality
Game & Parks Commission
University of Nebraska CES

Nevada

Department of Agriculture
Department of Agriculture, Entomologist
Division of Forestry
Division of Plant Industry, Department of Agriculture
Division of State Lands
Division of Wildlife
University of Nevada CES

New Hampshire

Belknap County CES
Cheshire County CES
Coos County CES
Department of Agriculture
Department of Resources & Economic Development
Division of Forests & Lands

Division of Plant Industry
Grafton County CES
Merrimack County CES
Natural Heritage Inventory
New Hampshire Fish & Game Department
Rockingham County CES
Strafford County CES
Sullivan County CES
University of New Hampshire CES
University of New Hampshire CES

New Jersey

Allaire State Park Nature Center
Atlantic County CES
Bergen County CES
Bergen County Park Commission
Boonton Township
Borough of Oakland
Bureau of Forest Management
Burlington County CES
Camden County CES
Cape May County CES
Cumberland County CES
Department of Agriculture
Department of Environmental Protection
Division of Fish & Wildlife
Division of Plant Industry
Essex County CES
Federation of Shade Tree Commission
Forestry Service
Glouchester County CES
Hunterdon County CES
Jersey City CES
Mercer County CES
Monmouth County CES
Monmouth City Park System
Monmouth County Shade Tree Commission
Morris County CES
Morris County Soil Conservation District
Mount Olive Township Council
New Jersey Division of Parks & Forestry
New Jersey Forest Tree Nursery
Ocean County CES

Parvi State Park
Passaic County CES
Pinelands Commission
Salem County CES
Somerset County CES
Somerset County Park Commission
Susses County CES
Union County CES
Warren County Soil Conservation District
Warren CES

New Mexico

Clauch-Pinto Soil & Water Conservation District
Department of Agriculture
Department of Agriculture, Bureau of Entomology &
     Nursery Industries
Department of Game & Fish
Energy Minerals & Natural Resource
Energy, Minerals & Natural Resource Department
Southwest New Mexico Council of Governments
State Forestry
State Land Office

New York

Albany County CES
Allehgany County CES
Broome County CES
Cayuga County CES
Chautaugua County CES
Chenango County CES
Clinton County CES
Clinton County Legislature
Columbia County CES
Cornell CES
Cortland County CES
County of Nassau Department of Recreation & Parks
Delaware County CES
Department Environmental Conservation
Department of Agriculture & Markets Division of Plant
     Industry
Department of Environmental Conservation Forest
     Resources Management

Department of Environmental Protection
Department of Health
Dutchess County CES
Essex County CES
Franklin County CES
Fulton County CES
Genesse County CES
Greene County CES
Herkimer County CES
Jefferson County CES
Lewis County CES
Livingston County CES
Monroa County CES
Montgomery County CES
Nassau County CES
New York State CES
Niagra County CES
New York Department of Environmental Conservation
    Urban Forest Coordinator
Oneida County CES
Onondaga County CES
Ontario County CES
Orange County CES
Orange County Department of Parks, Recreation, &
    Conservation
Orleans County CES
Oswego County CES
Otsego County CES
Pocono Forest & Wildlife Service
Putnam County CES
Rensselaer County CES
Rockland County CES
Saratoga County CES
Schenectady CES
Schoharie County CES
Seneca County CES
St. Lawrence County CES
State Department of Environmental Conservation
State Department of Agriculture
Steuben County CES
Suffolk County CES
Sullivan County CES
Tioga County CES

Tompkins County CES
Town of Granville
Ulster County CES
Warren County CES
Washington County CES
Wayne County CES
Westchester County CES
Wyoming County CES
Yates County CES

North Carolina

Carolina Department of Environment & Natural
Resources
Department of Agriculture & Consumer Services
Department of Agriculture Plant Protection Section
Division of Forest Resources
North Carolina CES
Plant Industry Division

North Dakota

Department of Agriculture
Forest Service
Game & Fish Department
State Historical Society of North Dakota

Ohio

Adams County CES
Allen County CES
Ashland County CES
Ashtabula County CES
Athen County CES
Auglaize County CES
Belmont County CES
Brown County CES
Butler County CES
Champaign County CES
Clark County CES
Clermont Couny CES
Clinton County CES
Colerain Township Parks
Columbiana County CES
Coshocton County CES

Crawford County CES
Cuyahoga County CES
Darke County CES
Defiance County CES
Deleware County CES
Division of Forestry
Erie County CES
Fairfield County CES
Fayette County CES
Franklin County CES
Fulton County CES
Gallia County CES
Geauga County CES
Geauga Park District
Guernsey County CES
Hamilton County CES
Hamilton County Park District
Hancock County CES
Hardin County CES
Harrison County CES
Henry County CES
Highland County CES
Hocking County CES
Holmes County CES
Huron County CES
Jackson Area Extension Center
Jackson County CES
Jefferson County CES
Knox County CES
Lake County CES
Lawrence County CES
Licking County CES
Logan County CES
Lorain County CES
Lucas County CES
Madison County CES
Mahoning County CES
Marion County CES
Medina County CES
Meigs County CES
Miami County CES
Monroe County CES
Montgomery County CES

Morgan County CES
Morrow County CES
Muskingum County CES
Noble County CES
Ohio Department of Agriculture
Ohio Department of Natural Resources
Ohio State University CES
Ohio State University Extension
Ottawa County CES
Paulding County CES
Perry County CES
Pickaway County CES
Pike County CES
Plant Pest Control Section, Department of Agriculture
Portage County CES
Preble County CES
Richland County CES
Ross County CES
Sandusky County CES
Scloto County CES
Seneca County CES
Shelby County CES
Stark/Summit CES
Trumball County CES
Tuscarawas County CES
Union County CES
Van Wert County CES
Vinton County CES
Warren County CES
Washington County CES
Williams County CES
Wood County CES
Wyandot County CES

Oklahoma

Department of Agriculture Plant Industries &
Consumer Services Division
Department of Agriculture, Forestry Services
Department of Environmental Quality
Department of Wildlife Conservation
Department of Agriculture
Putnam County CES

Oregon

CES Umatilla County
City of Eugene Public Works Department
City of Klamath Falls
Department of Agriculture
Department of Environmental Quality
Department of Fish & Wildlife
Department of Forestry
Department of Environmental Quality
Eugene Parks & Open Space
Fern Ridge Wildlife Area
Grant County & Harney County
Grant County Education Service District
Jackson County CES
Klamath Falls Resource Area
Lane County Environmental Health
Lane County Public Works
Lincoln County Health & Human Services
OSU Agriculture Chemistry Extension
Parks & Recreation Department
Portland Parks & Recreation
Umatilla Basin Watershed Council
Umatilla County Soil & Water
Union County Extension
Wasco County Weed Control

Pennsylvania

Adams County CES
Allegheny County CES
Armstrong County CES
Asbury Woods Nature Center
Beaver County CES
Bedford County CES
Berks County CES
Blair County CES
Bradford County CES
Bucks County CES
Butler County CES
Cambria County CES
Centre County CES
Chester County CES
Clarion County CES

Clearfield County CES
Clinton County CES
Commonwealth of Pennsylvania, Department of Environment
Crawford County CES
Cumberland County CES
Dams County CES
Dauphin County CES
Delaware County CES
Department of Conservation & Natural Resources
Elk County CES
Fayette County CES
Forest Pest Management
Four Mills Nature Reserve
Franklin County CES
Fulton County CES
Game Commission
Green County CES
Hawk Mountain Sanctuary
Huntingdon County CES
Indiana County CES
Jefferson County CES
Juniata City CES
Juniata County CES
Lackawanna County CES
Lancaster County CES
Lawrence County CES
Lebanon County CES
Lehigh County CES
Luzerne County CES
Lycoming City CES
Lycoming County CES
Mckean County CES
Mckean County CES
Mifflin County CES
Monroe County CES
Montgomery County CES
Montour County CES
Northhampton County CES
Northhumberland County CES
Pennsylvania Forest Stewardship Program
Perry County CES
Philadelphia County CES

Pike County CES
Potter County CES
Potter County CES
Schuylkill Conservation District
Schuylkill County CES
Snyder County CES
Stone Valley Recreation Area
Sullivan County CES
Susquehanna County CES
Tioga County CES
Union County CES
Venango County CES
Warren County CES
Washington County CES
Wayne County CES
Wayne County Gypsy Moth Coordinator
Westmoreland County CES
Wyoming County CES
York County CES

Rhode Island

Division of Agriculture & Resource Marketing
Division of Forest Environment
Jennings Nature Reserve
Rhode Island Department of Environmental
Management

South Carolina

Clemson University CES
Department of Natural Resource
Department of Plant Industry
Forestry Commission
Westvaco Forest Research Center

South Dakota

Department of Agriculture
Department of Environment & Natural Resources
Department of Game Fish & Parks

Tennessee

Department of Agriculture
Department of Agriculture - Division of Forestry

Department of Environment & Conservation
Department of Forestry, Wildlife & Fisheries
Department of Plant Industry
TN Agriculture CES
University of Tennessee Extension
Wildlife Resources Agency

Texas

Department of Agriculture
Forest Service
Parks & Wildlife Department

Utah

Department of Agriculture
Department of Natural Resources
Utah Department of Agriculture & Food Division of
    Plant Industry
Utah State University CES

Vermont

Department of Agriculture
Department of Agriculture Food & Markets Plant
    Industry Section
Department of Forest & Parks
Department of Forest & Parks Forest Resource
    Protection
Guildhall CES
Natural Resources Council
Newport Extension System
North Hero Extension System
Rutland Extension System
Skakel Forest Management
University of Vermont Extension
Vermont Agency of Natural Resources
Vermont Department of Fish & Wildlife
Vermont Extension System

Virginia

Alexandria Gypsy Moth Program
Bedford County CES
Botetourte County CES
CES

Clark County CES
Clark County Gypsy Moth Coordinator
Craig County CES
Culpeper County Gypsy Moth Coordinator
Department of Agriculture & Consumer Services
Department of Conservation & Recreation
Department of Forestry
Department of Game & Inland Fisheries
Dinwiddle County CES
Essex County CES
Fairfax County Gypsy Moth Program
Fairfax County Gypsy Moth Office
Flatwoods Civilian Conservation Center
Frederick County Gypsy Moth Program
Fulvanna County CES
Glocester County CES
Greensville County CES
Gypsy Moth Program Coordinator
Hanover County Gypsy Moth Program
Highland County Gypsy Moth Program
Isle of Wright County Gypsy Moth Program
King & Queen County CES
King County CES
King William County CES
Lancaster County CES
Louisa County Gypsy Moth Program
Madison County CES
Mathews County Gypsy Moth Program
Mecklenburg County CES
New Kent County CES
Northhampton County CES
Piedmont Environmental Council
Powhatan County Gypsy Moth Program
Prince Georges County CES
Prince William County Gypsy Moth Program
Prince William Forest Park
Rappahannock County CES
Richmond County CES
Roanoke County CES
Rockingham County Gypsy Moth Program
Rotetourt County CES
Shenandoah County CES
Sky Meadows State Park

Spotsylvania County CES
Surrey County CES
Virginia Polytechnic Institute (VPI) Extension Orange
   County
Warren County Gypsy Moth Program
Westmoreland County CES
York County Gypsy Moth Program

Washington

Alpine Lakes Protect Society
Bellevue Parks/Commission Service
Department of Ecology Office of Water Resources
Department of Fish & Wildlife
Department of Natural Resources
Department of Natural Resources, Northeast
Department of Wildlife Region 1 Habitat
Department of Agriculture Entomology
Evergreen State College
Glacier Public Service Center
Gonzaga University
King County Department of Natural Resources
King/Pierce County Farm Bureau
Natural Heritage Program
Northwest Wilderness Programs
Office of Archaeology & Historic Preservation
Okanogan Conservation District
Oregon Department of Environmental Quality
Seattle City Planning Office
State Department Agriculture
State Department of Agriculture Plant Services
   Division
State Department of Ecology
State of Washington, Department Natural Resources,
   Environmental Quality
Umatilla Forest Watch
Verlot Public Service Center
Washington Department of Natural Resources
   Southeast Region
Washington State Parks
Washington State University
Washington State University Department of Natural
   Resource Sciences

Washington State University, Extension
Washington Trout
Washington Wildlife Commission

West Virginia

Department of Agriculture
Department of Agriculture Plant Industries Division
Department of Energy
Department of Commerce, Labor & Environmental
    Resources
Division of Forestry
Hardy County Extension Agency
Harrison County CES
Home Health Agency of Davis Memorial Hospital
Plant Industries Program
State Lands Management
State Lands Management Division of Forestry
Upshur County
West Virginia Division of Natural Resources

Wisconsin

Adams County CES
Ashland County CES
Ashland County CES
Barron County CES
Barron County CES
Bayfield County CES
Brown County CES
Buffalo County CES
Burnett County CES
Calumet County CES
Chippewa City CES
Clark County CES
Columbia County CES
Crawford County CES
Dane County CES
Department of Agriculture
Department of Natural Resources
Department of Agriculture
Department of Agriculture & Trade
Devil's Lake State Park
Division of Forestry

Division of Health
Dodge County CES
Douglas County CES
Dunn City CES
Dunn County CES
Eau Claire County CES
Florence County CES
Fond Du Lac County CES
Forest County Board of Supervisors
Grant CES
Green County CES
Green Lake County CES
Iowa County CES
Iron County CES
Jackson County CES
Jefferson County CES
Juneau County CES
Kenosha County CES
Kewaunee County CES
Lacrosse County CES
Lafayette County CES
Langlade County Board of Supervisors
Langlade County Forestry Department
Layfayette County Board
Lincoln CES
Lincoln County Forestry Land & Park Departmen
Manitowo CES
Manitowoc County Soil & Water Conservation
Marathon County CES
Marinett County CES
Marquette County CES
Menomine County CES
Milwaukee County CES
Mondovi Township
Monroe County CES
Oconto County CES
Oneida County CES
Oneida County Board
Outagamie County CES
Ozaukee County CES
Pepin County CES
Pierce CES
Plant Industry Bureau

Polk County CES
Port Washington CES
Portage CES
Price County CES
Racine County CES
Richland CES
Rock County CES
Rock County Park & Conservation Division
Rusk County CES
Sauk CES
Shawano CES
Sheboygan CES
St. Croix CES
Taylor County CES
Town of Brooklyn
Town of Caledonia
Town of Jump River
Town of Knox
Town of Newbold
Town of Rhine
Town of Schoepre
Town of Spring Prairie
Town of Troy
Trempealeau County CES
Trenton Township
University of Wisconsin CES
Vernon County CES
Vilas County CES
Vilas County Community Resource
Walworth CES
Washburn County CES
Washington CES
Waukesha CES
Waupaca County CES
Winnebago CES
Winnebago County CES
Wisconsin Department of Agriculture
Wisconsin Department of Natural Resources
Wood CES
Wood County

Wyoming

Department of Agriculture
Department of Agriculture Technical Services Division
Department of State Parks, & Cultural Resources
Game & Fish Department
State Forestry Division
State Lands & Investments
University of Wyoming CES

Puerto Rico

University of Puerto Rico Agriculture Experiment Station

Canada

BC Ministry of Agriculture & Food
Ontario Forestry Association

6.3 American Indian Nations, Tribes, and Related Agencies

Alabama

Poarch Creek Indians

Alaska

Atmautluak Traditional Council
Andreafski Tribal Council
Agdaagux Tribe of King Cove
Akiachak Native Community
Akiak Native Community
Akutan Traditional Council
Alatna Tribal Office
Aleut Community of St. Paul Island
Algaaciq Tribal Council
Allakaket Village
Angoon Community Association
Anvik Tribal Council
Asa'carsarmiut Tribe
Beaver Tribal Council
Birch Creek Village
Central Council Tlingit & Haida Indian Tribes
Chalkyitsik Village

Chefornak Traditional Council

Chenega Council

Chevak Traditional Council

Chickaloon Village Traditional Council

Chignik Lake Traditional Village Council

Chilkat Indian Village (Klukwan)

Chilkoot Indian Association

Chinik Eskimo Community (Aka Golovin)

Chitina Traditional Village

Chuloonawick Native Village

Circle Native Community

Craig Community Association

Crooked Creek Traditional Council

Curyung Tribal Council

Dot Lake Village Council

Douglas Indian Association

Egegik Village

Ekwok Village Council

Emmonak Village

Evansville Tribal Council

Gulkana Village

Healy Lake Traditional Council

Holy Cross Tribal Council

Hoonah Indian Association

Hughes Village Council

Huslia Village Council

Hydaburg Cooperative Association

Igiugig Village

Inupiat Community of Arctic Slope

Iqurmuit Tribe (Russian Mission)

Ivanoff Bay Village Council

Kaguyak Village

Kaktovik Village

Kenaitze Indian Tribe

Ketchikan Indian Corporation

King Island Native Community

Klawock Cooperative Assn

Knik Tribe

Kobuk Traditional Council

Kokhanok Village

Kongiganak Traditional Council

Koyukuk Native Village

Kwigillingok Council

Larsen Bay Tribal Council

Lesnoi Village

Levelock Village

Lime Village

Louden Tribal Council

Manley Hot Springs Village

Manokotak Village

Mary's Igloo Traditional Council

Mcgrath Native Village Council

Mentasta Traditional Tribal Council

Metlakatla Indian Community

Naknek Native Village Council

Native Village Nuiqsut

Native Village of Afognak

Native Village of Akhiok

Native Village of Aleknagik

Native Village of Ambler

Native Village of Atqasuk

Native Village of Barrow Inupiat Traditional

Native Village of Belkofski

Native Village of Brevig Mission

Native Village of Buckland

Native Village of Cantwell

Native Village of Chignik

Native Village of Chignik Lagoon

Native Village of Chistochina

Native Village of Chuathbaluk

Native Village of Council

Native Village of Deering

Native Village of Diomede (Ira) (Aka Inalik)

Native Village of Eagle

Native Village of Eek

Native Village of Eklutna

Native Village of Ekuk

Native Village of Elim

Native Village of Eyak

Native Village of False Pass

Native Village of Fort Yukonnative

Native Village of Gakona

Native Village of Gambell

Native Village of Georgetown

Native Village of Goodnews Bay

Native Village of Hamilton

Native Village of Hooper Bay
Native Village of Kanatak
Native Village of Karluk
Native Village of Kasaan
Native Village of Kasigluk
Native Village of Kiana
Native Village of Kipnuk
Native Village of Kivalina
Native Village of Kluti-Kaah (Aka Copper Cent)
Native Village of Kotzebue
Native Village of Koyuk
Native Village of Kwinhagak
Native Village of Marshall (Aka Fortuna Ledge)
Native Village of Minto
Native Village of Nanwalek (Aka English Bay)
Native Village of Napaimute
Native Village of Napakiak
Native Village of Napaskiak
Native Village of Nikolski
Native Village of Noatak
Native Village of Nunapitchuk
Native Village of Ouzinkie
Native Village of Paimiut
Native Village of Pauloff Harbor
Native Village of Perryville
Native Village of Pitka's Point
Native Village of Point Hope
Native Village of Port Graham
Native Village of Port Heiden
Native Village of Port Lions
Native Village of Savoonga
Native Village of Scammon Bay
Native Village of Shaktoolik
Native Village of Sheldon Point
Native Village of Shishmaref
Native Village of Shungnak
Native Village of South Naknek
Native Village of St. Michael
Native Village of Stevens
Native Village of Tanacross
Native Village of Tanana
Native Village of Tatitlek
Native Village of Tetlin

Native Village of Tuntutuliak
Native Village of Tununak
Native Village of Tyonek
Native Village of Unalakleet
Native Village of Venetie Tribal Government
Native Village of Wales
Native Village of White Mountain
Nelson Lagoon Tribal Council
Nenana Native Association
New Koliganek Village Council
New Stuyahok Village
Newhalen Tribal Council
Newtok Traditional Council
Nightmute Traditional Council
Nikolai Village
Ninilchik Village Traditional Council
Nome Eskimo Community
Nondalton Village
Noorvik Native Community
Northway Village,
Nulato Village
Nunakauyak Traditional Council
Organized Village of Grayling
Organized Village of Kake, Petersburg Indian
Association
Organized Village of Kwethluk
Organized Village of Saxman
Orutsararmuit Native Council
Oscarville Tribal Council
Pedro Bay Village
Pilot Point Traditional Council
Pilot Station Traditional Village
Platinum Traditional Village Council
Portage Creek Village
Qagan Tayagungin Tribe
Qawalangin Tribe of Unalaska
Rampart Village
Ruby Tribal Council
Selawik Ira Council
Seldovia Village Tribe
Shageluk Native Village
Sitka Tribe of Alaska
Skagway Village

Sleetmute Traditional Council
Solomon Traditional Council
Stebbins Community Association
Takotna Tribal Council
Tazlina Village Council
Telida Native Village Council
Teller Traditional Council
Traditional Village of Togiak
Tuluksak Native Community
Twin Hills Village Council
Ugashik Traditional Village Council
Umkumiut Native Village
Unga Tribal Council
Village of Alakanuk
Village of Anaktuvuk Pass
Village of Aniak
Village of Arctic Village
Village of Clarks Point
Village of Iliamna
Village of Kalskag
Village of Kaltag
Village of Kotlik
Village of Lower Kalskag
Village of Ohogamiut
Village of Old Harbor
Village of Point Lay
Village of Red Devil
Village of Salamatof
Village of Stony River
Village of Wainwright
Wrangell Cooperative Assn
Yakutat Tlingit Tribe

Arizona

Cocopah Tribal Council
Havasupai Tribal Council
Hopi Tribal Council
Hualapai Tribal Council
Pascua Yaqui Tribal Council
San Juan Southern Paiute Council
Tohono O'odham Nation
White Mountain Apache Tribal Council
White Mountain Apache Tribe

California

Agua Caliente Band of Cahuilla Indians
Alturas Rancheria
Augustine Band of Mission Indians
Barona Band of Mission Indians
Bear River Band of Rohnerville Rancheria
Benton Paiute Reservation
Berry Creek Rancheria
Big Lagoon Rancheria
Big Pine Reservation
Big Sandy Rancheria
Big Valley Rancheria
Bishop Reservation
Blue Lake Rancheria
Bridgeport Indian Colony
Buena Vista Rancheria
Cabazon Tribal Business Committee
Cahuilla Band of Mission Indians
Campo Band of Mission Indians
Cedarville Rancheria
Chemehuevi Tribal Council
Chicken Ranch Rancheria
Cloverdale Rancheria
Cold Springs Rancheria
Colusa Rancheria
Cortina Rancheria
Cuyapaipe Band of Mission Indians
Dry Creek Rancheria
Elem Indian Colony
Elk Valley Rancheria
Enterprise Rancheria
Fort Bidwell Reservation
Fort Independence Reservation
Fort Mojave Tribal Council
Greenville Rancheria
Grindstone Rancheria
Guidiville Rancheria
Hoopa Valley Tribal Council
Hopland Reservation
Inaja-Cosmit Reservation
Ione Band of Miwok Indians
Jackson Rancheria
Jamul Indian Village

Karuk Tribe of California
La Jolla Band of Luiseno Indians
La Posta Band of Mission Indians
Laytonville Rancheria
Lone Pine Reservation
Los Coyotes Reservation
Lytton Rancheria
Manchester - Point Arena Rancheria
Manzanita Band of Mission Indians
Mechoopda Indian Tribe of the Chico Rancheria
Mesa Grande Band of Mission Indians
Mooretown Rancheria
Morongo Band of Mission Indians
Pala Band of Mission Indians
Paskenta Band of Nomlaki Indians
Pauma/Yuima Band of Mission Indians
Pechanga Band of Mission Indians
Picayune Rancheria
Pinoleville Reservation
Pit River Tribal Council
Potter Valley Rancheria
Quartz Valley Reservation
Ramona Band of Mission Indians
Redding Rancheria
Redwood Valley Reservation
Resighini Rancheria
Rincon Band of Mission Indians
Robinson Rancheria
Round Valley Reservation
Rumsey Rancheria
San Manuel Band of Mission Indians
San Pasqual Band of Diegueno Indians
Santa Rosa Band of Mission Indians
Santa Ynez Band of Mission Indians
Santa Ysabel Band of Mission Indians
Scotts Valley Rancheria
Sheep Ranch Rancheria
Sherwood Valley Rancheria
Shingle Springs Rancheria
Smith River Rancheria
Soboba Band of Mission Indians
Stewart Point Rancheria
Susanville Indian Rancheria

Sycuan Band of Mission Indians
Table Bluff Reservation
Table Mountain Rancheria
Torres-Martinez Desert Cahuilla Indians
Trinidad Rancheria
Tule River Reservation
Twenty-Nine Palms Band of Mission Indians
United Auburn Indian Community
Upper Lake Rancheria
Viejas Band of Mission Indians
Woodfords Community Council
Yurok Tribe, Middletown Rancheria

Colorado

Southern Ute Tribe
Ute Mountain Ute Tribe

Connecticut

Mashantucket Pequot Tribe
Mohegan Indian Tribe

Florida

Miccosukee Indian Tribe
Seminole Indian Tribe

Iowa

Sac & Fox Tribe of the Mississippi in Iowa

Kansas

Iowa Tribe of Kansas & Nebraska
Kickapoo Tribe in Kansas
Prairie Band Potawatomi Indians
Sac & Fox Tribes

Louisiana

Chitimacha Indian Tribe
Coushatta Indian Tribe
Jena Band of Choctaw Indians
Tunica-Biloxi Tribe

Maine

Aroostook Band of Micmacs
Houlton Band of Maliseet Indians
Passamaquoddy Tribe
Penobscot Indian Nation
Penobscot Nation Department of Natural Resources

Massachusetts

The Trustee of Reservations
Wampanoag Tribe of Gay Head (Aquinnah)

Michigan

Bay Mills Indian Community of Michigan
Grand Traverse Band of Ottawa & Chippewa
Hannahville Indian Community of Michigan
Huron Potawatomi, Inc.
Keweenaw Bay Indian Community of Michigan
Lac Vieux Desert Band of Lake Superior Chippewas
Little River Band of Ottawa Indians
Little Traverse Bay Bands of Odawa Indians
Match-E-Be-Nash-Wish Band of Pottawatomi Indians
Pokagon Band of Potawatomi Indians of Michigan
Saginaw Chippewa Indian Tribe of Michigan
Sault Ste. Marie Tribe of Chippewa Indians

Minnesota

Bois Forte Reservation Business Committee
Fond Du Lac Reservation
Fond Du Lac Reservation Business Committee
Grand Portage Reservation Business Committee
Leech Lake Reservation
Leech Lake Reservation Business Committee
Lower Sioux Indian Community of Minnesota
Mille Lacs Band of Chippewa Indians
Mille Lacs Reservation Business Committee
Minnesota Chippewa Tribe
Prairie Island Indian Community of Minnesota
Red Lake Band of Chippewa Indians
Red Lake Band of Chippewa Indians of Minnesota
Shakopee Mdewakanton Sioux Community of
    Minnesota

Upper Sioux Community of Minnesota
White Earth Reservation Business Committee

Mississippi

Mississippi Band of Choctaw Indians

Montana

Blackfeet Tribal Business Council
Chippewa Cree Business Committee
Confederated Salish & Kootenai Tribes, Tribal
Crow Tribal Council
Fort Belknap Community Council
Fort Peck Tribal Executive Board
Northern Cheyenne Tribal Council

Nebraska

Omaha Tribal Council
Ponca Tribe of Nebraska
Santee Sioux Tribal Council
Winnebago Tribal Council

Nevada

Battle Mountain Band Council
Carson Community Council
Dresslerville Community Council
Duckwater Tribal Council
Elko Band Council
Ely Colony Tribal Council
Fallon Paiute Shoshone Tribal Business Council
Fort Mcdermitt Tribal Council
Las Vegas Tribal Council
Lovelock Tribal Council
Moapa Business Council
Pyramid Lake Paiute Tribal Council
Reno-Sparks Tribal Council
Shoshone-Paiute Business Council
South Fork Band Council
Stewart Community Council
Summit Lake Paiute Council
Te-Moak Tribe of Western Shoshone
Walker River Paiute Tribal Council
Washoe Tribal Council

Wells Indian Colony Band Council
Winnemucca Tribal Council
Yerington Paiute Tribe
Yomba Tribal Council

New Mexico

Jicarilla Apache Tribe
Mescalero Apache Tribe
Pueblo of Acoma
Pueblo of Cochiti
Pueblo of Isleta
Pueblo of Jemez
Pueblo of Laguna
Pueblo of Nambe
Pueblo of Picuris
Pueblo of Pojoaque
Pueblo of San Felipe
Pueblo of San Ildefonso
Pueblo of San Juan
Pueblo of Sandia
Pueblo of Santa Ana
Pueblo of Santa Clara
Pueblo of Santo Domingo
Pueblo of Taos
Pueblo of Tesuque
Pueblo of Zia
Pueblo of Zuni
Ramah Navajo Chapter

New York

Blue Mtn. Reservation
Cayuga Nation of Indians
Oneida Indian Nation
Onondaga Indian Nation
Seneca Nation of Indians
St. Regis Mohawk Tribe
Tonawanda Band of Seneca
Tuscarora Nation

North Carolina

Eastern Band of Cherokee Indians

North Dakota

Spirit Lake Tribal Council
Standing Rock Sioux Tribal Council
Three Affiliated Tribes Business Council
Turtle Mountain Band of Chippewa

Oklahoma

Absentee-Shawnee Tribe of Oklahoma
Alabama-Quassarte Tribal Town
Apache Tribe of Oklahoma
Caddo Indian Tribe of Oklahoma
Cherokee Nation of Oklahoma
Cheyenne-Arapaho Tribes of Oklahoma
Chickasaw Nation
Choctaw Nation of Oklahoma
Citizen Potawatomi Nation
Comanche Indian Tribe
Delaware Tribe of Indians
Delaware Tribe of Western Oklahoma
Fort Sill Apache Tribe of Oklahoma
Iowa Tribe of Oklahoma
Kaw Tribe of Oklahoma
Kialegee Tribal Town
Kickapoo Tribe of Oklahoma
Kiowa Tribe of Oklahoma
Miami Tribe of Oklahoma
Modoc Tribe of Oklahoma
Muscogee (Creek) Nation
Osage Tribal Council
Otoe-Missouria Tribe of Oklahoma
Ottawa Tribe of Oklahoma
Pawnee Tribal Business Council
Peoria Tribe of Oklahoma
Ponca Tribe of Oklahoma
Quapaw Tribal Business Committee
Sac & Fox Nation of Oklahoma
Seminole Nation of Oklahoma
Seneca-Cayuga Tribe of Oklahoma
Thlopthlocco Tribal Town
Tonkawa Tribe of Oklahoma
United Keetoowah Band of Cherokee Indians

Wichita & Affiliated Tribes
Wyandotte Tribe of Oklahoma

Oregon

Burns Paiute Tribe, General Council
Confederated Tribes of Coos, Lower Umpqua
Confederated Tribes of the Grand Ronde Commun
Confederated Tribes of the Umatilla Indian
Confederated Tribes of the Warm Springs Reservation
Coquille Indian Tribe
Klamath General Council
Siletz Tribal Council

Rhode Island

Narragansett Indian Tribe

South Carolina

Catawba Indian Tribe

South Dakota

Cheyenne River Sioux Tribe
Crow Creek Sioux Tribal Council
Flandreau Santee Sioux Executive Committee
Lower Brule Sioux Tribal Council
Oglala Sioux Tribal Council
Rosebud Sioux Tribal Council
Sisseton-Wahpeton Sioux Tribal Council
Yankton Sioux Tribal Business & Claims Committee

Texas

Alabama-Coushatta Tribe of Texas
Kickapoo Traditional Tribe of Texas
Ysleta Del Sur Pueblo

Utah

Goshute Business Council
Paiute Indian Tribe of Utah Tribal Council
Skull Valley Band of Goshutes
Uintah & Ouray Tribal Business Committee

Washington

Colville Business Council
Confederated Tribes of the Chehalis
Hoh Tribal Business Committee
Jamestown S'klallam Tribal Council
Kalispel Business Committee
Lower Elwha Tribal Council
Lummi Indian Business Council
Makah Indian Tribal Council
Muckleshoot Tribal Council
Nez Perce Contact
Nisqually Indian Community Council
Nooksack Indian Tribal Council
NW Indian Fisheries Commission
Port Gamble S'klallam Tribe
Puyallup Tribal Council
Quileute Tribal Council
Quinault Indian Nation - Business Committee
Samish Indian Nation
Sauk-Suiattle Tribal Council
Shoalwater Bay Tribal Council
Skokomish Tribal Council
Snoqualmie Tribal Organization
Spokane Business Council
Squaxin Island Tribal Council
Suquamish Tribal Council
Swinomish Indian Tribal Community
Umatilla Forest Resource Council
Upper Skagit Tribal Council
Yakama Indian Nation

Wisconsin

Bad River Band of Lake Superior Chippewa Indians
Forest County Potawatomi
Forest County Potawatomi Community of Wisconsin
Great Lakes Intertribal Council
Ho-Chunk Nation
Lac Courte Oreilles Band of Lake Superior Chippewas
Lac Du Flambeau Band of Lake Superior Chippewas
Lac Du Flamebeau Tribal Natural Resource Depa
Menominee Indian Tribe of Wisconsin
Menominee Tribal Enterprises

Oneida Tribe of Indians of Wisconsin
Red Cliff Band of Lake Superior Chippewa Indians
Sokaogon Chippewa (Mole Lake) Community)
Sokaogon Chippewa Community
Sokaogon Chippewa Tribe
St. Croix Chippewa Indians of Wisconsin
St. Croix Chippewa Tribe
Stockbridge Munsee Community of Wisconsin
Stockbridge-Munsee Community
Stockbridge-Munsee Tribe
Wisconsin Winnebago Business

Wyoming

Arapaho Business Committee
Shoshone Business Committee

6.4 Organizations

Alabama

Alabama Nursery Association
Auburn University CES
Auburn University, Department of Entomology & Plant Pathology
B.A.S.S., Inc.

Alaska

Alaska Conservation Alliance
Alaska Defenders of Wildlife
Alaska Forest Association
Alaska Rainforest Campaign
Alaskan Society of Forest Dwellers
Alaskans for Responsible Resource Management
Cook Inlet Region, Inc.
Copper River Delta Institute
Friends of Berners Bay
Friends of the Earth
Greenpeace
Rural Advisor Office of the Governor
Society of American Foresters Alaska
Tongass Conservation Society

Arizona

Arizona for Wildlife Conservation
Arizona Nature Conservancy
Arizona Wilderness Coalition
Arizona Wildlife Federation
Audubon Society, Huachuca
Audubon Society, Northern Section
Audubon Society, Tucson
Center for Biological Diversity
Citizens for Protection of Prescott Arizona
Citizens of Mt. Graham Scientific Council
Cochise Conservation Council
Conservation Chair
Defenders of Wildlife
Ecological Restoration Institute
Environmental Services, Phelps Dodge Morenci, Inc.
Environmental Services
Flagstaff Activist Network
Fort Apache Timber Co.
Foundation for Biodiversity
Grand Canyon Trust
Hebbard & Webb, Inc.
Maricopa Audubon Society
Native Plant Society of Arizona
The Nature Conservancy
North Country, Inc.
Northern Arizona Loggers Association
Northwest Pine Products
Phoenix Zoo
Plateau Group
Prescott Forest Friends
Rincon Group People for the West
Rocky Mountain Elk Foundation
Roosevelt Community Association
Salt River Project
Scentry Biologicals, Inc.
Sierra Club
Sierra Club, Grand Canyon Chapter
Sky Island Alliance
Southern Arizona Environmental Council
Sonoran Biodiversity Project,
Southwest Environmental Center
Southwest Forest Alliance

Southwest Forest Watch
Trout Unlimited
Verde Watershed Association
White Mountain Conservation League
The Wilderness Society, Arizona Chapter
Wildlife Society, Arizona Chapter

Arkansas

Arkansas Forestry Association
Defenders of Quachita Forest
Ozark Organic Growers Association

California

Alternatives to Toxics
American Land Conservancy
American River Conservancy
Aspen Environmental Group
Bear Engineering
California Forestry Association
California Native Plant Society
California State Polytechnic University
California State University - Sacramento
Conservation Congress
Council for Planning & Conservation
Earthjustice, Headquarters
Entrix
Five Creek Limited Partnership
Forest Landowners of California
Golden Queen Mining Co.
Greystone
Humboldt State University, Department of Forestry
Natural Resources Defense Council
Oregon Heirs Corp.
PG&E Corporation
Preservation Officer, Oregon-California Trails
    Association
Robert Burt & Rebecca Burt Family Trust
Santa Cruz Rainforest Action Group
Sierra Club
Society for the Protection & Care of Wildlife
Talon Associates
Trinity River Lumber Co.

University of California, Department of Ecology &
    Evolution
University of San Diego, Pardee Legal Research Center
University of the Pacific

Colorado

Burns & McDonnell
Colorado Forestry Association
The Denver Gold Group
KTUN News Director
Meet the Wilderness
Native American Fish & Wildlife Society
Rocky Mountain Bird Observatory
Sierra Club, Rocky Mountain Chapter (Colorado)
Wilderness Society
Wildlife Management Institute

Connecticut

Club, Connecticut Chapter
Connwood Foresters, Inc.
Connecticut Forests & Park Association
Dubois Forestry Land Management
Forestland Associates
Hull Forestland Management
Keep America Beautiful
Sierra Conwood, Inc.
Tamarack Tree Co.
Timberline Management Co.
University of Conneticut CES
Yale School of Forestry

Delaware

Air Enterprises, Inc.
College of Agriculture & Sciences University of
Delaware
Delaware Campground Owners Association
Delaware Nature Education Society
Delaware Nature Society
Delaware State University
Department of Agriculture & Natural Resources
Dover Post
Drake Farms

Hopeland Farms
Stafford Homeowners Association
T. S. Smith & Sons, Inc.
The Cedars Academy
University of Delaware Entomology

District of Columbia

American Forest Council
American Forest Resource Alliance
American Forests
American Lands Alliance
American Paper Institute
American Pulpwood Association
American Recreation Coalition
American Rivers
American Ski Federation
Americans for the Environment
Association of State & Territorial Health Officers
Coalition for Scenic Beauty
Council of Governors' Policy
Defenders of Wildlife
Ecological Society of America, The
Endangered Species Coalition
Friends of the Earth
George Washington University Library
Global Leleaf
Greenpeace USA
International Association of Fish & Wildlife
National Agricultural Chemical Association
National Association of Conservation District
National Association of Counties
National Association of State Foresters
National Audubon Society
National Coalition Against the Misuse of Pesticides
National Conference of State Legislatures
National Council for Science & the Environment
National Forest Products Association
National Governors Association
The National Grange
National Parks Conservation Association
National Tree Trust
National Urban League
Natural Resources Council of America

Natural Resources Defense Council
Public Health Foundation
Public Lands Council
Save America's Forests
Sierra Club, Washington DC Office
Sport Fishing Institute
U.S. Public Interest Research Group
United States Tourist Council
Urban Forestry Administration
Western Governors Association
Wilderness Society, The
World Resources Institute
World Wildlife Fund

Florida

Florida A&M University
Florida Forestry Association
Florida International University
Florida Native Plant Society
Florida State University
Forest Management Trust
Great Outdoors Conservancy
St. Thomas University
Tree Advisors
University of Central Florida
University of Florida, School of Forest Resources
University of South Florida, Sarasota
University of South Florida, Tampa
University of Tampa
University of West Florida

Georgia

Atlanta Audubon Society
Augusta State University
Berry College
Columbus State University
Dalton State College
Emory University
Forest Landowners Association, Inc.
Forest Watch Coordinator
Fox Forestry, Inc.
Georgia Organic Growers Association

Georgia College & State University

Georgia Department of Natural Resources

Georgia Federation of Forest Owners

Georgia Forestry Association, Inc.

Georgia Institute of Technology

Georgia Southern University

Georgia Southwestern State University

Georgia State University

Kennesaw State University

Mercer University

National Forest Products Association

North Georgia College & State University

Savannah Tree Foundation

Sierra Club, Georgia Chapter

Southeast Lumber Manufacturers Association

State University of West Georgia

Toxic Commission & Assistance Project

Trees Atlanta

Union Camp Woodlands Corp.

University of Georgia

University of Georgia, Department of Entomology

Valdosta State University

Warnell School of Forest Resources

Hawaii

Brigham Young University - Hawaii

University of Hawaii - Manoa

Idaho

Albertson College of Idaho

Association Logging Contractors, Inc.

Bioanalysts

Blue Ribbon Coalition

Boise Cascade Corporation

Boise State University

Caldwell R. & E. Center

Carney Products Co. Ltd

Ceda-Pine Beneer

CH2M Hill

Citizens for a User Friendly Forest (CUFF)

Coeur D'Alene Chamber of Commerce

Coeur D'Alene Tribal Forestry

College of Forestry University of Idaho

Croman Corp

Crown Pacific Island

Dames & Moore

Economic Modeling Specialists

Flying Resort Ranches

Friends of the Clearwater

Hells Canyon Alliance

Idaho Audubon Council

Idaho Conservation League

Idaho Forest Industries, Inc.

Idaho Nursery & Landscape

Idaho Power Co.

Idaho State University

Idaho Trails Council

Idaho Water Users Association

Idaho Watersheds Project

Idaho Wildlife Federation

Idaho Women in Timber

Intermountain Forest Industry Association

JAG, Inc.

Kootenai Environmental Alliance

Lewis-Clark State College

Ling, Nielsen & Robinson

Louisiana-Pacific Corporation

Northwest Management, Inc.

Northwest Timber Workers Resourse Council

Potlatch Corporation

President, ID Chapter, Oregon California Trails
    Association (OCTA)

Regulus Stud Mills, Inc.

Resource Solutions

Ricks College

Selkirk-Priest Basin Association

Sierra Club

Society of American Foresters, Intermountain

Spokesman-Review

St. Joe Economic Development Foundation

Three Rivers Timber, Inc.

University of Idaho Department of Forest Resources-
    Entomology

University of Idaho

University of Idaho Extension Forestry

University of Idaho Forest, Wildlife & Range Policy
    Analysis Group
University of Idaho, College of Forestry, Forest
    Entomology
University of Idaho Society of American Foresters
Western Forest Environmental Alternative
Western States Equipment
Wilderness Society

Illinois

Abbot Laboratories Capd
America Defender Network
American Nurseyman
Benedictine University
Bradley University
Capital Agriculture Property Service, Inc.
Chicago Region Biodiversity Council, Chicago
Chicago State University
Depaul University
Dominican University
Eastern Illinois University
Governors State University
Harold Flying Service
Hendrickson Flying Service
Illinois Nurserymen's Association
Illinois Forest Products Co., Inc.
Illinois Native Plant Society
Illinois State Library
Illinois State University
Illinois Valley Community College
Illinois Walnut Council
Illinois Wesleyan University
International Society of Arboriculturists
John A. Logan College
Lake Forest College
Lewis University
Loyola University, Chicago
Monmouth College
Moraine Valley Community College
National Campers & Hikers Association
North American Wildlife Foundation
Northeastern Illinois University
Northern Illinois University

Northwestern University
Northwestern University School of Law
Olivet Nazarene University
Open Lands Project
Plant Illinois
Pontiac Flying Service
Race & Heartwood
Reed's Fly-On Farming
Rocky Moutain Elk Foundation
Southeast Illinois College
Sierra Club Great Lakes Chapter
South Suburban College
Southern Illinois University Carbondale
Tri-State Forestry
University of Chicago
University of Illinois Extension, University
University of Illinois, Chicago
University of Illinois at Urbana, Champaign
Urbana Forestry Management, Inc.
Valent Biosciences Corp
Western Illinois University
Wheaton College
Wildlife Society Illinois Chapter

Indiana

Ace Pest Control
Akard Forestry Consultants
Al's Aerial Spraying
Anderson University
Ball State University
Berg-Warner Nursery, Inc.
C.S. Bond Forest Management
Chris Leibering & Sons
Depaul University
Forest & Land Managers, Inc.
Forest Management Services
Hanover College
Hensler Nursery, Inc.
Hopwood Forestry Consultants
Hudson Forestry Co.
Huntington College
Indiana Forestry & Woodland Owners Association
Indiana State University

Indiana University
Indiana University, Kokomo
Indiana University, Northwest
Indiana University, South Bend
Indiana University, Southeast
Indiana Wood Products, Inc.
Pike Lumber Co., Inc.
Purdue University Hardwood Tree Improvement &
    Regeneration Center
Purdue University
Purdue University Entomology Department
Saint Joseph College
Schuerman Forestry Service
Stambaugh Forestry & Nursery
University of Notre Dame
University of Southern Indiana
Valparaiso University
Wabash College
Walley Lumber Co.
Walnut Council
Weston Paper

Iowa

Control Services
Cornell College
Department of Entomology Iowa State University
Drake University
Drake University
Geode Resource Conservation and Development
(RC&D)
Grinnell College
Haugen Contracting
Iowa State University
Iowa State University Department of Plant Pathology
Iowa State University Extension Forester
Iowa State University Extension Horticulture
Iowa State University of Science & Tech.
Iowa Woodland Owners Association
Izaak Walton League of America Endowment
Krambeer Forestry Services, Inc.
Lone Tree Nursery
Murphy's Walnut Hill Nursery
National Association of County Engineers

North Iowa Area Community College
Northwest Landscaping, Inc.
Northwestern College
Pathfinders RC&D
Pella Nurseries
Peters Logging & Milling
Soil & Water Conservation Society of America
State Library of Iowa
Tiedt Nursery & Forestry Service
Trees Forever
Trees-R-Us
University of Iowa
University of Northern Iowa
Upper Iowa University
West Enterprises

Kansas

Baker University
Colby Community College
Dodge City Community College
Fort Hays State University
Kansas City Community College
Kansas State University
Kansas Wesleyan University
University of Kansas
Washburn University
Wichita State University

Kentucky

Department of Entomology, University of Kentucky
Eastern Kentucky University
Kentucky State University
Kentucky Wesleyan College
Kentucky Woodland Owners Association
Kentucky Forest Industries Association
Morehead State University
Murray State University
Northern Kentucky University
Union College
University of Kentucky
University of Kentucky Department of Entomology
University of Kentucky Department of Forestry

University of Louisville
Western Kentucky University

Louisiana

Alexandria Forestry Center
Louisiana College
Louisiana Forestry Association
Louisiana State University Department of Entomology
Louisiana State University, Baton Rouge
Louisiana State University, Eunice
Louisiana State University, Shreveport
Louisiana Tech University
Loyola University, New Orleans
Norrie Colony, Inc.
Northwestern State University
Southeastern Louisiana University
Southern Forest Products Association
Southern University A&M College
Tulane University
University of Louisiana, Lafayette
University of Louisiana, Monroe
Xavier University of Louisiana

Maine

American Pulpwood Association, Inc.
Andrews Land Service, Inc.
Bates College
Bear Paw Lumber
Bowdoin College
Champion International
Colby College
Coolong Land Surveys
Eco- Analysts
Edgewood Tree Farm
Evans Notch Visitor Center
Forest Society of Maine
Forests by Design
International Paper Co.
The Ireland Group
James River Timber
James W. Sewall Co.
Landvest, Inc.

Leonardi Associates
Llavalley Lumber Co.
M.S. Lavoie Air
Mackintosh Forest Management Services
Maine Maritime Academy
Maple Hill Forest Services
Marine Helicopters
Marty's Logging
National Audubon Society, Maine Audubon
Natural Resources Council of Maine
Prentiss & Carlisle Co., Inc.
S.D. Warren Woodlands
Sierra Club, Maine Chapter
Small Woodland Owners Association of Maine
Southern Maine Forestry Services
Timberland Consultants
Two Trees Forestry
University of Maine, School of Law
University of Maine, College of Natural Sciences
University of Maine, Orono
University of Maine, Presque Isle
Whittling Ridge Farm
Wood Fiber Industries
Woodlot Alternatives, Inc.
Woodlot Management Services

Maryland

Allegany College of Maryland
Alliance for Maryland Forest
American Hiking Society
Associationiatoin of Consulting Foresters America,
Center for Watershed Diversity
Chesapeake Corporation
Chesapeake Forest-Land Services
East Coast Helicopter, Inc.
Entomological Society of America
Forestry Concepts
Frostburg State University
Glatfelter Pulp Wood Co.
Helicopters Applications, Inc.
International Society of Tropical Foresters
Izaak Walton League of America, Inc.
James Bailey Agrotors

Kennedy Consultants
The Land & Tree Co.
Maryland Campground Owners Association
Maryland Chistmas Tree Association
Maryland Forest, Park & Wildlife Service
Maryland Forests Association
Maryland Native Plant Society
Michel Forestry Co.
National Military Fish & Wildlife Association
The Orchards Association, Inc.
Parker Forestry Services
Parkton Woodland Services
Pickering Creek Audubon Center, Audubon Maryland
Pine Top Woodland Improvement Ser.
Piney Run Nature Center
Rachel Carson Council, Inc.
Renewable Natural Resources Foundation
Salisbury State University
Society of American Foresters
Spicer Lumber Co.
University of Baltimore
University of Maryland at College Park
University of Maryland, Baltimore County
Versar, Inc.
Washington College
Western Maryland College
Wildlife Habitat Council
Wood Products, Inc.

Massachusetts

Amherst College
Appalacian Mountain Club
Beaver Tree Work
Boston Athenaeum Library
Boston College
Boston University, School of Law
Brandeis University
Conservation Law Foundation of Northeast
Earthwatch
Forest Logic
Forester - W.D. Cowle, Inc.
Gordon College
Harvard College

Holdsworth Natural Resource Center
The Land Concern, Inc.
Massachusetts Audobon Society
Massachusetts Forestry Associatione
Massachusetts Institute of Technology
National Parks Conservation Association
New England Forestry Foundation
North American Family Campers Association
Northeastern University
Sierra Club, Massachusetts Chapter
Trust for Public Land
Tufts University
Tufts University Environmental Program
Turnagain Resources
University of Massachusetts, Amherst
University of Massachusetts CES
University of Massachusetts, Dartmouth
University of Massachusetts, Department of
    Entomology
University of Massachusetts, Department of Forestry &
    Wildlife Mgmt
University of Massachusetts Extension
University of Massachusetts, Lowell
University of Massachusetts, Medical Center
University of Massachusetts, Shade Tree Lab
University of Massachusetts, Western MA Agriculture
    Center
University of Massachusetts, Department of Zoology
Wellesley College
Western New England College
Williams College

Michigan

Abitibi-Price Corporation
Albion College
Al's Aerial Spraying
American Motorcycle Association
American Society of Agricultural Engineers
Armintrout's Nursery
Battelle Great Lakes Environmental Center
Biewer Sawmill, Inc.
Big Creek Associates
Central Michigan University

Champion Fleet Owners Association

Citizens for Alternatives to Chemical Contamination

Consumers Power Co.

Cycle Conservation Club of Michigan

Delta College

Dow Chemical Co.

Earl's Spray Service, Inc.

Eastern Michigan University

The Ecology Center

Federation Natural Resources

Federation of Fly Fishers

Ferris State College

Ferris State University, Department of Bio Science

Forest-Land Services, Inc.

Georgia Pacific Corporation

Global Relief of Michigan

Great Lakes Camp & Trail Association

Grosse Pointe Woods Tree Commission

Hatfields Spraying Service

Huron Audobon Club

Hydrolake Leasing & Sales

Keweenaw Land Association, Ltd.

Lake States Forestry Alliance

Lake States Lumber Association

Lake Superior State University

Mackinac Chapter - Sierra Club

Marble Institute of America

Maurice's Flying Service

Mead - Publishing Paper Division

Metropolitan Forestry Consultants, Inc.

Michigan Association of Private Campground Owners

Michigan Association of Timbermen

Michigan Audubon Society

Michigan Bow Hunters

Michigan Chamber of Commerce

Michigan Conservation Foudation

Michigan Environmental Council

Michigan Environmental Defense League

Michigan Forest Products Industry Dev. Council

Michigan Forests Association

Michigan Independent Wood Products Association

Michigan Nature Association

Michigan Nature Association

Michigan Recreation Canoe Association

Michigan Salmon & Steelheader Association

Michigan Snowmobile Association

Michigan State University Department of Entomology

Michigan State University Extension

Michigan State University, Pesticide Research Center

Michigan Tech University, School of Forestry & Wildlife

Michigan Technological University

Michigan Technological University School of Forest & Wood Products

Michigan Trail Riders Association

Michigan Tree

Michigan Trout Unlimited

Michigan United Conservation Clubs

Michigan Wild Turkey Federation

Michigan Wilderness Prevention

Michigans Trapper's Association

Mid-Michigan Helicopters, Inc.

Morth County Trail Association

Michigan State University, Department of Forestry

National Gypsy Moth Management Group

National Woodlands

Nature Conservancy

North Central Michigan College

North Country Trail Association

Northeast Michigan Sportsmen Club

Northern Hardwoods

Northern Michigan University

Northwestern Financial Center

Oakland University

Oscoda Sierra Club

Outdoor Access, Inc.

Packaging Corporation of America

Pere Marquette Watershed Council

Pictured Rocks National Lakeshore

Potts Tree Farm

Ruffed Grouse Society

Screamin Eagle Aviation

Sierra Club

Sierra Club, West Michigan Group

Steelhead Anglers

Steiger Lumber Co.

Timberwatch
Trout Unlimited
University of Michigan, Botanical Gardens
University of Michigan, School of Natural Resources
West Michigan Environmental Action Council
West Michigan Tourist Association
Western Michigan University
Weyerhauser
Wiggins Tree Co.
Wild Turkey Federation
The Wilderness Society

Minnesota

Airborne Custom Spraying, Inc.
Cook Co.
Forest Management Specialists, Inc.
Forestry Associates
Hamline University
Heartwood Forestry
Kunde Co. Forestry
Minnesota Forestry Association
Minnesota Ornithologists' Union
Moorhead State University
Mountland Timber, Inc.
North Hardwoods Notes
Plant Health Associates, Inc.
Potlatch Corp.
Privatelands Forestry Consulting
Rijala Timber Co.
Saint Cloud State University
Split Rock Forestry, Inc.
Sundance Silviculture
Two by Forestry
Woodland Services, Inc.

Mississippi

Alcorn State University
Delta State University
Jackson State University
Missisipe State University Department of Entomology
Mississippi College
Mississippi Department of Agriculture & Commerce

Mississippi Forestry Commission
Mississippi State University Department of Forestry
Mississippi State University, College of Forestry
Mississippi University for Women
Mississippi State University
Mississippi Forestry Commission
National Association of Professional Forestry Schools
Sidney Malone International
University of Mississippi
University of Southern Mississippi

Missouri

Audubon Society of Missouri
Central Missouri State University
Chamberlain & De James
Department of Conservation/Forestry Division
Dowler's Lower Place
Foremost Forest Managers
G-W Lumber Co.
Hammons Products Co.
Jefferson College
Lincoln University
Lindenwood University
Loners of America
Maryville University of Saint Louis
Meers & Associates
Metropolitan Forestry Services
Missouri Forest Products Association
Missouri Native Plant Society
Missouri Southern State College
Missouri Forest Management Co.
National Timber Consultants
Northwest Missouri State University
Port Hudson Timber Management
Rockhurst University
Saint Louis University
Schnurbusch Land Services
Sierra Club, Ozark Chapter (Missouri)
Skip Kincaid & Associates
Southeast Missouri State University
Southeastern Association of Fish & Wildlife Agencies
Southwest Missouri State University
Steward Agriculture Research Services

Truman State University
University Extension, University of Missouri
University of Missouri
University of Missouri, Columbia Department of
   Entomology
University of Missouri, Columbia Department of
   Entomology
University of Missouri, Columbia
University of Missouri, Kansas City
University of Missouri, Rolla
University of Missouri, Saint Louis
University of Missouri, School of Forestry, Fisheries &
   Wildlife
Washington University
Washington Wheatley Neighborhood Association
William Jewell College

Montana

Alliance for the Wild Rockies Ecosystem Defense
American Wildlands
Belt Creek Information Center
CS & KT Forestry
Lewis & Clark Intrepretive Center
Missoula Technology Development Center
Montana Forest Owners Association
Montana State University, Billings
Montana State University, Bozeman
Montana State University, Northern
Montana Tech/University of Montana
National Audubon Society, Montana Audubon
National Forest Foundation
Rocky Mountain Elk Foundation
Scentry Biologicals., Inc.
University of Montana
Wilderness Watch

Nebraska

Creighton University
Dana College
National Arbor Day Foundation
University of Nebraska, Kearney

University of Nebraska, Lincoln
University of Nebraska, Omaha
Wayne State College

Nevada

University of Nevada, Las Vegas
University of Nevada, Reno

New Hampshire

Blue Hills Forest Products
Dartmouth College
Foreco
Gorham Land Co.
James River Corp.
Little Pro Timber Service
New Hampshire Association of Conservation Districts
New Hampshire College
New Hampshire Landowners Alliance
New Hampshire Timberland Owners Association
NH Timberlands Owners Association
Northern Forest Lands
Preserve Appalachian Wilderness
Saint Anselm College
Sierra Club, New Hampshire Chapter
Society for the Protection of New Hampshire Forests
Trust for New Hampshire Lands
University of New Hampshire
Urban Forestry Center
Wagner Woodlands, Inc.
Waterville Valley Co.
White Mountain Attractions Association
Wonalancet Outdoor Club

New Jersey

Aeolium Nature Center
Alliance for Environmental Concerns
American Littoral Society
Arbor Management
Batsto Nature Center
Bergab County Wildlife Center
Cattus Island Nature Center
Center for Environmental Studies

College of Saint Elizabeth
Conservation & Environmental Center, Inc.
Consolidated Eastern Corp.
Cordoba Helicopter Enterprises, Inc.
Cornucopia Network of New Jersey, Inc.
County College of Morris
The Delicate Balance
Downstown Aero Crop Service, Inc.
Drew University
Fairleigh Dickinson University
Flat Rock Brook, Director
Forest Management Services
Forestry Section, Cook College
Galway Forestry Services
Grassroots Environmental Organization
H & S Forestry Co., Inc.
The Hope Commission
Interstate Pest Control Compact
Irvington Outdoor Education Center
Monmouth University
Montclair State University
New Jersey Association of Conservation Districts
New Jersey Beekeepers Association, Inc.
New Jersey City University
New Jersey Coalition for Alternatives to Pesticides
New Jersey Conservation Foundation
New Jersey Environmental Federation
New Jersey Forestry Association
Ocean County College
Palisades Nature Association
Paul Cowie Associates
PGE
Poricy Park Nature Center
Princeton Education Center at Blairstown
Princeton University
Public Service Electric & Gas Co.
Rdg Associates, Inc.
Rider University
Rowan University
Rutgers University, Camden
Rutgers University, New Brunswick
Rutgers University, Newark
Sandy Hook Environmental Education Center

Seton Hall University
Seton Hall University, School of Law
Shade Tree Commission
Sierra Club Chatam
South Branch Watershed Association
Spermaceti Cove Visitor Center
Stony Brook-Millstone Watershed
Tenafly Nature Center
Trailside Nature & Science Center, Director
Upper Raritan Watershed Association
Watergate Environmental Education Center
Weis Ecology Center
The Wetlands Institute
YMCA Camp Bernie

New Mexico

Audubon Society
Audubon Society, Central New Mexico
Audubon Society, Southwest New Mexico
Carson Forest Watch
Chippeway Lumber, Inc.
Coalition for Public Lands & Natural Resources
Coalition of AZ/NM Counties
Conklin Lumber Co
Department of Natural Science, WNMU
Earth First, New Mexico
Eastern New Mexico University
Forest Conservation Council
Forest Guild
Forest Trust
Forestry Association, Inc.
Gila Conservation Coalition
Gila Watch
Hansen Lumber Co., Inc.
Hawkwatch International
Izaak Walton League
Kuykendall Lumber Co.
La Jicarita Enterprise, Inc.
Madera Forest Products Coop
MCS Task Force of NM
Moore Cash Lumber
Native Plant Society of New Mexico
The Nature Conservancy of New Mexico

New Mexico Audubon Council
New Mexico Earth First!
New Mexico Farm & Livestock Bureau
New Mexico Highlands University
New Mexico Junior College
New Mexico Public Interest Research Group
New Mexico Public Land Council
New Mexico Rural Development Response
New Mexico State University
New Mexico Wilderness Study Committee
People for the West
Public Land Users Association
The Quivira Coalition
Sanchez Timber & Mill Co.
Santa Fe Canyon Association
Santa Fe Forestry Council
Scientech
Sierra Club
Sierra Club, Albuquerque Group
Sierra Club, Pajarito Group
Sierra Club, Rio Grande Chapter
Sierra Club, Santa Fe Chapter
Sierra Club, Tularosa Basin Group
Taos Birders
Taos Nature Society
University of New Mexico
University of New Mexico, School of Law
Western Environmental Law Center
Western Network
Western New Mexico University
Wild Turkey Federation, Las Cruces Chapter
Zuni River Watershed

New York

A to Z Forestry
Adelphi University
Adirondack Conservancy
Adirondack Council
Adirondack Eco-Center
Adirondack Forestry, Inc.
Adirondack Mountain Club
Airspray, Inc.
Alley Pond Enviroment Center, Inc.

Alpine Forestry
American Birding Association, Inc.
American Forest Council
American Nature Study Society
American Whitwater Affiliation
Appalachian Forestry Consulting Services
Arbor Care Ltd.
Arthur W. Butler Memorial Sanctuary
Ashokan Field Campus
Baltimore Woods
Bayard Cutting Arboretum
Beaver Lake Nature Center
Beaversprite Nature Center
Binghamton University
Brooklyn Botanic Garden
Brooklyn College/College University of New York
    (CUNY)
Brooks Resources Management Co.
Buttermilk Falls Tree & Turf, Inc.
Camp Greenkill Environmental Education Center
Camp Owahta Outdoor Education Center
Capake Falls State Park Nature Center
Cary Arboretum
Catskill Forest Association
Catskill Mountain Forestry Service
Catskill Real Estate Appraisals
Christian Gearwar
City College/CUNY
Clarkson University
Clear Creek Consulting
Colgate University
The College of Insurance
Columbia University
Conservation Advisory Committee
Cooper Union
Co-operating Consultant Foresters
Cornell Laboratory of Ornithology
Cornell University
Cornell University CES
Cornell University, Department of Entomology
The Cummings Nature Center
Delaware Valley Forestry Service
Downing Enviroment & Forest Consultant

Dyken Pond Environment Education Center

Earth First

East-West Forestry Association

Elmira College

Empire State Forestry Service

Environmental Action Coalition

Environmental Defense, Headquarters

Erie County CES

Ferncliff Forest

Five Rivers Environmental Education Center

Fordham University

Fordham University, School of Law

Forecon, Inc.

Forest-All: Tree & Forest Care

Forsite Forestry

Fountain Forestry, Inc.

Golden Valley Outdoor Recreation Center

Great Neck Outdoor Environmental Center

Green Chimneys Farm Center

Greenburg Nature Center

Greenwood Park

Gunlocke Co.

Heldeberg Workshop

Herbert H. Lehman College/CUNY

High Rock Park Conservation Center

Hillside Outdoor Education Center

Hofstra University

Intermountain Forestry

Intermountain Forestry

International Paper Co.

Inwood Hill Park Environmental Education Center

IPM Laboratories

Kenneth L. Willimas & Association

Long Island University

Mallery Lumber Co.

Manitoga Hudson River Nature Center

Mianus River Gorge Wildlife Refuge & Botanica

Micha Tree & Landscape Consultants

Miller Forest Products

Minna Anthony Com. Nat. Center

Monroe Tree & Landscape, Inc.

Muscoot Park Interp. Farm

Museum of Hudson Highlands

Nassau County Museum Preserve

National Audubon Society

National Campers & Hikers Association

National Council of Paper Industry

Natural Resources Defense Council, Inc.

North East State Foresters Association

New York Botanical Gardens Institute of Ecosystem Studies

New York Forest Owners Association

New York Law Institute

New York Law School

New York University

New York Zoological Park

Northeast Timber Services

Northern Consulting

Northern States Tree Services, Inc.

Norton Timberland Management

NY Department of Environmental Cons.

Oceanside Marine National Study Area

Pace Environmental Center

Peterson Forestry, Inc.

Pioneer Forestry Service

Planting Fields Arboretum

Plattsburgh State University

Pok-O-Moonshine Outdoor Education Center

Queens Botanical Garden

Queens College/CUNY

Quogue Wildlife Refuge

Regional Plan Association

Rockland Lake Nature Center

Rudolf Steiner Farm School

Rye Nature Center

Sackhoes Region Nature Center

Saint Bonaventure University

Saint John's University

Sapsucker Woods Bird Sanctuary

Sarah Lawrence College

Sharpe Enviroment Center

Skidmore College

South Fork, Shelter Island Chapter

St. Lawrence University

State University College of Technology

State University College of Brockport

State University College of Cortland
State University College of Geneseo
State University College of New Paltz
State University College of Oneonta
State University College of Oswego
State University College of Potsdam
State University of New York (SUNY)
SUNY, Albany
SUNY, Buffalo
SUNY, College of Environmental Sciences & Forestry
SUNY, Farmingdale
SUNY Institute
SUNY Maritime College
SUNY, Stony Brook
SUNY, Department of Natural Resources
Sylvan Forestry Services
Synecology Forest Management
Syracuse University
T. Roosevelt Memorial Bird Sanctuary
Teatown Lake Reservation
Thompson Pond Project
Thorington Forestry Service
Timberland
Town of North Salem
Town of Warrensburg
Trailside Nature Museum
Trust for Public Land
Twin Valleys Outdoor Education Center
Union College
University of Rochester
Upland Farm
Upper Delaware Council
Upper Delaware Scenic & Rec. River
Vassar College
Wave Hill Center for Environ. Study
Weinberg Nature Center
Westmoreland Sanctuary, Inc.
Winding Hills Park Nature Center

North Carolina

Abw Lumber Indiustries, Inc.
Apex Nurseries, Inc.
Appalachian State University

Associationiated Hardwoods, Inc.
Bartlett Tree Rsch Lab
Beard, E.N. Hardwood Co.
Campbell University
Catawba College
Cramer Lumber Co.
Davidson College
Duke University
Duke University School of Forestry & Environmental
  Studies
East Carolina University
Fayetteville State University
Forest History Society, Inc.
Gilkey Lumber Co., Inc.
Interforest, Inc.
Mount Olive College
National Foundation for the Chemically Hypersensitive
National Toxics Campaign
NC Christmas Tree Association
North Carolina A & T State University
North Carolina Central University
North Carolina Forestry Association
North Carolina State University
North Carolina State University College of Forest
  Resources
North Carolina State University Department of
  Entomology
North Carolina Wesleyan College
North Carolina Wildlife Resources Commission
Oaks Unlimited
Prime Lumber Co.
Queens College
Southern Appalachian Biodiversity Project
University of North Carolina, Chapel Hill
University of North Carolina, Charlotte
University of North Carolina, Greensboro
University of North Carolina, Pembroke
University of North Carolina, Wilmington
Voohees & Pitts Lumber Co., Inc.
Wake Forest University
Watson Lumber Co., Inc.
Western Carolina University

North Dakota

North Dakota State University
Sully's Hill-N-Game Preserve
University of North Dakota

Ohio

American Farm Tree Program
Ashland University
Bob Ruhe AG Service
Bowling Green State University
Capital University
Case Western Reserve University
Cleveland State University
College of Wooster
Custom Forestry
Denison University
Downing Woodland Services
Forest Resource Consultants
Hooking College
International Association of Natural Resource
John Carroll University
Johnson's Forest Products
The Longaberger Co.
Kent State University
Kenyon College
MacArthur Lumber & Post Co.
Marietta College
Marketing Labs Co., Inc.
Meadow Woodlands
Med Woodlands
Miami University
Miami University - Middletown
Municipal Arborists & Urban Foresters Society
Muskingum College
Native Plant Society of Northeast Ohio
Oberlin College
Ohio Christmas Tree Association, Inc.
Ohio Ecological Food & Farm Association
Ohio Forestry Association, Inc.
The Ohio Lepidopterists
Ohio Integrated Pest Management Program
Ohio Northern University

Ohio Nursery Association
Ohio State University
Ohio State University Extension Lucas Co.
Ohio Tree Consulting Services
Ohio University
Ohio Wesleyan University
Olde Forester Consultants
Otterbein College
Potts Tree Farm
Prime Air
Shawnee State University
Society of Municipal Arborists
Timberland Forestry Consutants
Tree Sentry
Treevalue Forestry Service
University of Akron
University of Cincinnati
University of Dayton
University of Findlay
University of Toledo
Wright State University
Youngstown State University

Oklahoma

East Central University
National Watershed Coalition
Native Americans for a Clean Environment
Northeastern State University
Northwestern Oklahoma State University
Oklahoma Forestry Association.
Oklahoma State University
Oklahoma State University Department of Forestry
Oklahoma Woodland Owners Association
Southeastern Oklahoma State University
Southern Nazarene University
Southwestern Oklahoma State University
University of Central Oklahoma
University of Oklahoma
University of Tulsa

Oregon

Andersen Forestry Consulting
Agri-Pacific Resources, Inc.
Alice V. Wissman Living Trust
American Fisheries Society - Oregon Chapter
American Lands Alliance
Argus Observer
Associated Oregon Loggers
Atterbury Consultants, Inc.
Audubon Society of Portland
Avion Water Co., Inc.
B & S Logging, Inc.
Bark
Big Pines RV Park
Black Butte Resort
Blue Mountain Back Country Horsemen
Blue Mountain Biodiversity Project
Blue Mountain Native Forest Alliance
Blue Mountains Biodiversity Project
Blue Mountain Lumber Products
Boise Cascade Corporation
Boise Corporation of Northeast Oregon
Brandt-Nelson, Lark & Brandt
The Bulletin
Camp Tamarack
Capital Press
Cascadia Forest Alliance
Center for Environmental Equity
Central Cascades Alliance
Central Oregon Audubon Society
Central Oregon Community College
Central Oregon Small Woodlands Associates
Central Point Lumber
Chambers Communication Corp
Churchill, Leonard, Brown, Lodine, & Hendrie
Circle De Lumber Co.
City of Eugene, Parks & Open Space
Clouston Energy Research
Cold Springs Resort
Columbia Basin Fish and Wildlife Authority
Concerned Friends ofthe Winema
Consolidated Pine
Crane Prairie Resort

Crescent Creek Cottages
Crescent Lake Lodge & Resort
Crescent Lake RV Park
Crescent Water Association
Crown Pacific Ltd.
Crown River Corp. & Crown Zellerbach
Cultus Lake Resort
D.R. Johnson Lumber (Prarie Wood Products)
David Evans & Associates
Defenders of Wildlife
Deschutes Province Advisory Committee
Double-D-Logging
Douglas Timber Operators, Inc.
Dow Agrosciences
Dr. Johnson Lumber Companies
Dunn Family Trust
Earth Share of Oregon
Eastern Oregon University
Eastern Oregon University Baker Center
Eco-Northwest
Ecola Creek Awareness Project
Ecosystem Equity Council
Emerald Chapter, Native Plant Society of Oregon
Environmental Studies Center
Eugene Burrill Lumber Co.
Eugene Parks & Open Space
Evergreen Helicopters
Forest Recovery, Inc.
Forest Resource Management, Inc.
Forest Resource Services
Forest Service Employees for Environmental Ethics
Fowler Timber Co.
Friends of the Columbia Gorge
Friends of Black Butte Ranch
Friends of Living Oregon Waters
Friends of the Greensprings
Friends of the Metolius
Friends of the Metolius-Environmental Advocates
Glide Lumber Co./Western Timber Co.
Grande Ronde Resource Council
Grande Ronde Model Watershed Program
Grant County Conservationists
Grizzly Mountain Aviation

Guistina Resources
Haglund, Kirtley, Kelly & Horngren LLP
Halfway House Gallery & Gifts
Headwaters
Hells Canyon Preservation Council
Henderson Logging
High Desert Committee, Sierra Club
Hood River County Weed & Pest Director
Independent Forest Products Association
Institute for Fisheries Resources
Izaak Walton League
J. & J. Logging
J. Herbert Stone Nursery
Joseph Timber Co.
K/P Corporation
Keerins Ranch
Kinzua Resources
Klamath Siskiyou Wildlands Center
L. & D., Inc.
Lane County Audubon Society
Lapine Forestry Services, Inc.
Larch Co.
Lewis & Clark College
Lewis & Clark Law School
Linfield College
Lowell Service Center
Lowell Work Center
M. & L. Enterprises
Malheur Lumber Co.
Malheur Timber Operators, Inc.
Mason, Bruce & Girard, Inc.
Mckenzie Guardians
Metolius Meadows Property Owners Association, Inc.
Metolius Recreation Association
Metolius River Summer Homes
Mid-Columbia Native Plant Society
Monarch Magic
Mountaineers
Mt. Bachelor, Inc.
Musselman & Association, Inc.
Myrmo & Sons, Inc.
National Audubon Society
Native Plant Society

Native Plant Society of Oregon, Blue Mountain
    Chapter
Native Plant Society of Oregon, Wm Cusick Chapter
The Nature Conservancy
Neighborhood Association
Net Work Association Ecological Consulting
North American Butterfly Association
North American Wild Sheep
North Santiam Watershed Council
North Santiam Watershed Forum
Northwest Coalition for Alternatives to Pesticides
Northwest Environmental Defense Council
Northwest Forestry Association
Northwest Resource Council
Northwest Special Forest Products Association
Northwestern School of Law
The Nugget Natural Areas Association
Ochoco Lumber Co.
Odell Lake Homeowners Association
Odell Lake Resort
Odell Sportsman
Old Cascades Wilderness Com
One World Trade Center
Oregon Association of Nurserymen
Oregon Cattlemen's Association
Oregon Eagle Foundation
Oregon Equestrian Trails
Oregon Farm Bureau
Oregon Forest Homeowners Association
Oregon Hunters Association
Oregon Natural Desert Association
Oregon Natural Resources Council
Oregon Poison Center
Oregon Rivers Council
Oregon Sierra Club Wildlands
Oregon Small Woodlands Association
Oregon Society of American Foresters
Oregon State Public Interest Research Group
Oregon State Snowmobile Association
Oregon State University
Oregon State University CES
Oregon State University, Department of Botany &
    Plant Pathology

Oregon State University, Department of Entomology
Oregon State University, Department of Forest
    Management
Oregon State University, Department of Rangeland
    Resources
Oregon State University Integrated Plant Protection
    Center, Cordley Hall
Oregon Tilth, Inc.
Oregon Water Resources
Ouzel Outfitters
P & M Lumber & Cedar Products
Pacific Environmental Advocacy Center (PEAC)
Pacific University
Pacificorp
The Pacific Rivers Council
Pape Brothers, Inc.
Pendleton Record
People for the West
Pine Creek Logging, Inc.
Pine Point Forest
Portland State University
Portland General Electric
Prarie Wood Products
Public Forestry Foundation
Reed College
Rei Co-Op
River Conservancy
Robert E. Morris Contracting
Rogue Valley Audubon Society of Medford
Rosboro Lumber Co.
Rosebud Contracting
Ross Trust
Salmon-Drift Creek Watersheds Group
Samuel S. Johnson Foundation
Sandy River Basin Watershed Council
Save Our Wild Salmon Coalition
Save the West
Shirbeck, Inc.
Sierra Club
Sierra Club Juniper Group
Sierra Club Oregon Chapter
Sierra Club Portland
Sierra Club, Oregon Chapter

Siskiyou Regional Educational Project
Sisters Forest Planning Committee
Smith Properties
Society Advocating Natural Ecosystems
Society for Range Management
Society of American Forersters, OR State Society
South Oregon University- Biology Department
Southeast Oregon Resource Advisory Council
Southern Oregon University
Steens Mountain Packers
Sun Mountain Water Systems, Inc.
Sunriver Nature Center
Sunriver Owners Association
Sunriver Properties Oregon Ltd.
Sunriver Resort
Thompson Timber Co.
Timber Data Co
Trout Unlimited-Oregon Council
Twin Lakes Resort
Twin Rivers Logging Co.
Umatilla Basin Watershed
University of Oregon
Upper Deschutes Watershed Council
Wagon Wheel Water Co.
Walla Walla Basin Watershed Council
Wallowa Forest Products (D.R. Johnson)
Washington Forest Law Center
Water Wonderland Improvement District
Western Ancient Forest Campaign
Western Environmental Law Center
Western Farm Service
Western Forestry & Conservation Association
Western Oregon University
Western Radio Services Co, Inc.
Wilderness Trail Riders, Inc.
Wildland Resources
Wildlife Management Institute
Wildlife Society, Oregon Chapter
Willamette Industries
Willamette Pass Inn
Willamette University
Woodsman Motel
Xerces Society

Yaquina Basin Planning Team
Zacharias Logging

Pennsylvania

A.D. Renninger Lumber Co.
Agrotors
Ahora Tree Service
Alder Valley Forestry Consulting
Allegheny Acres
Allegheny College
Allegheny Foresters & Consultants
Allegheny Forestry, Inc.
Allegheny Portage Railroad
American Forestry Consultants
Andrews Woodlot Consulting Service
Appalachian Forest Consutants
Arboreal Forestry Services
Bailey Lumber Co.
Bear Run Nature Reserve
Beechwood Farms Nature Reserve
Bloomsburg University of Pennsylvania
Bradford Area Chamber of Commerce
Bradford Naturalist Club
Briar Bush Nature Center
Brownlee Lumber, Inc.
Bucknell University
C.F.E., Inc.
Carl Hunsberger Sawmill, Inc.
Chas. M. Shaffer Memorial Natl. Center
Clifford B. Carts Co.
Coastal Lumber Co.
Collins Pine Kane Hardwood Division
Columbia County CES
Derwood Nature Center
Duquesne University
Dwight Lewis Lumber Co.
East Stroudsburg University
Felton Associates
Forest Land Services, Inc.
Forest Management Center
Forest Management Associates, Inc.
Forest/Woodlot Management, Inc.
Forestry & Wildlife Consulting

Franklin & Marshall College
Franklin Foresty Services
Freeman's Forestry & Wildlife Services
Fulton Forest Products
Glatfelter Pulpwood Co.
Haverford College
Hercon Environmental
Highlands Lumber Co., Inc.
Hyma-Devore Lumber Co.
Indiana University of Pennsylvania
International Paper Co.
J.M. Wood Products, Inc.
Jay-For Logging
John J. Tyler Arboretum
Joseph W. Arnold Association
Kane Hardwood Division, Collins Pine
Kuhns Bros. Lumber Co., Inc.
La Roche College
Lamar National Wildlife Refuge
Landon Forestry Services
Lapp Lumber Co.
Lehigh University
Longwood Gardens
Mansfield University
Meiser Lumber Co.
Miller Ag-Craft, Ltd
Miller Aircraft Limited
Millersville University of Pennsylvania
Montgomery County Community College
Mooretown Mill
Morris Arboretum
Mt. Valley Farms & Lumber Products, Inc.
Muhlenberg College
Nagy & Webb Forestry & Surveying Services
National Audobon Society, Audobon Science off
National Gypsy Moth Management
National Gypsy Moth Management Group, Inc.
Northern Timber Services
Northern Timberlands
Open Land Conservancy
Penelec Manager of Forestry
Penn Forestry Co., Inc.
Penn State New Kensington

Pennsylvania Deer Association
Pennsylvania Forestry Association
Pennsylvania State University
Pennsylvania State University Department of
    Entomology
Polaris Surveying & Forestry
Proctor & Gamble Paper Co.
Pennsylvania State University, School of Forest
    Resources
Ram Forest Products
Regional Vice President National Audubon Society
Robert Labar Forestry Consultant
Robert Morris College
Robert S. Bommer, Jr., Inc.
Rohm & Haas
Rolling Rock Farms
The Ruffed Grouse Society
Saint Joseph's University
Seneca Highlands Association
Shippensburg University of Pennsylvania
Sierra Club, Pennsylvania Chapter
Slippery Rock University
Sparty-Wood Products, Inc.
Sunderland Surveying & Forestry
Susquehanna County Historical Society & Free Library
    Association
Swarthmore College
Tallman Aerial Spraying
Temple University
Timber Mgmt Services
Tinicum National Environmental Center
Twin Ponds Sawmill
Twin Tier Systems
University of Pennsylvania
University of Pittsburgh
University of Pittsburgh-Bradford
Valent Biosciences
Villanova University.
Walter H. Weaver Co,
West Chester University Department of Biology
West Chester University of Pennsylvania
Wheeland Lumber Co., Inc.
Whites Wood Nature Center

Rhode Island

Brown University
Florence Gray Center
Group for Alternative to Spraying Pesticides
National Network of Forest Practitioners
Pesticide Public Policy Foundation
Providence College
Rhode Island College
Rhode Island Forest Conservator's Organization
Rhode Island Organic Farmer's Association
Southern New England Forest Consortium, Inc.
Southern Northeast Woodland Service
Turnquist Lumber Co.
University of Rhode Island
University of Rhode Island CES
University of Rhode Island, Department of Plant
    Sciences
University of Rhode Island, Department of Forestry

South Carolina

Center for Forested Wetlands
Charleston Southern University
Clemson University
Clemson University, Department of Forest Resources
Coastal Carolina University
College of Charleston
Francis Marion University
Furman University
Lander University
South Carolina State University
Southern Appalachian Botanical Society
University of South Carolina, Aiken
University of South Carolina, Columbia
University of South Carolina, Lancaster
Winthrop University

South Dakota

Black Hills State University
Northern State University
South Dakota State University
University of South Dakota

Tennessee

Austin Peay State University
Cleveland State Community College
Columbia State Community College
East Tennessee State University
Fisk University
Hardwood Forest Foundation
Hardwood Research Council
King College
Lambuth University
Middle Tennessee State University
Sierra Club, Tennessee Chapter
Tennessee Forestry Association
Tennessee State University
Tennessee Technological University
United States Aviation Underwriters
University of Memphis
University of Tennessee, Department of Forestry,
    Wildlife & Fisheries
University of Tennessee, Knoxville
University of Tennessee, Martin
University of the South
Vanderbilt University

Texas

Angelo State University
Audubon Society
Bat Conservation International
Baylor University
F. Austin State University
Geo-Marine, Inc.
Hardin-Simmons University
Howard Payne University
Lamar University
Loma Linda Homeowners Association
Midwestern State University
Mitchell Energy Corp.
Navarro College
Rice University
Saint Mary's University
Sam Houston State University
San Antonio College

Sierra Club, El Paso Regional Group
South Texas College of Law
Southern Methodist University
Southwest Texas State University
Stephen Texas Forestry Association
Tarrant County College
Texarkana College
Texas A&M International University
Texas A&M University
Texas A&M University Research & Extension Center
Texas A&M University, Commerce
Texas A&M University, Corpus Christi
Texas A&M University, Kingsville
Texas Christian University
Texas Southern University
Texas Tech University
Trinity University
University of Houston
University of Houston, Clear Lake
University of Houston, Victoria
University of North Texas
University of Texas, Arlington
University of Texas, Austin
University of Texas, Dallas
University of Texas, El Paso
University of Texas, Pan American
University of Texas, San Antonio
West Texas A&M University

Utah

Brigham Young University
Southern Utah University
University of Utah
University of Utah, Department of Biology
Utah State University
Utah Woodland Owners Council
Vermillion Services
Weber State University
Western Association of Land Users

Vermont

Harwood Forestry Services
Nature's Light
Society of American Foresters New England
Upland Resource Group, Inc.
Vermont Law School

Virginia

American Pulpwood Association
Association of Consulting Foresters of America
Chespeake Forest Products Co.
Citizens for a Better America
College of William & Mary
Conservation Foundation
Dupont Nature Club
Dynamic Aviation
Emory & Henry College
Friendly Forest Farms
Future Farmers of America
George Mason University
Hampton University
Helicopter Association International
Highland County CES
Hollins University
James Madison University
Labot-Anderson
Madison County Library, Inc.
Mary Washington College
National Campground Owners Association
National Recreation & Parks Association
National Wildlife Federation
National Wildlife Federation, Headquarters
National Woodland Owners Association
Nature Conservancy
Old Dominion University
Old Time Orchard
Potomac Appalachian Trail Club
Roanoke College
Tetrotech
Trout Unlimited
University of Richmond
University of Virginia

University of Virginia's College at Wise
Virginia Polytechnic Institute & State University,
    Department of Entomology
Virginia Commonwealth University
Virginia Forestry Association
Virginia Native Plant Society
Virginia Polytechnic Institute
Virginia State University
Virginia Tech University, College of Natural Resources
Virginia Tech University, Department of Entomology
Virginia Tech University, Department of Fisheries &
    Wildlife
Virginia Tech University, Department of Forestry
Virginia Wesleyan College
Virginians for Wilderness/Earth First
Washington & Lee University

Washington

49 Degrees North Ski Area
Alpine Lakes Protect Society
Alps Trustee
Alpine Lakes Protection Society Alta Crystal Resort,
    L.L.C.
American Land Rights Association
American Lands Access Association, Inc.
American Rivers
Apple Valley Broadcasting
Auble Association
Audubon Society, North Cascades
Audubon Society, Skagit Chapter
Backcountry Bicycle Trails
Backcountry Horsemen of Washington, Inc.
Backpacker Magazine
Bear Creek Tree Farms
Bellingham Mountaineers
The Bloedel Reserve
Camp Sheppard, BSA - Chief Seattle Chapter
Center for Environmental Law & Policy
Central Washington University
Central Washington University Department of Geog. &
    Land Studies
Chinook Byways
Citizens of Greenwater

Coalition for a Livable Washington
Columbia Basin Nursery
Columbiana
Common Sense Resource League
Concerned Friends of Ferry County
Crown Zellerbach Corp.
Crystal Mountain Resort
David Evans & Associates
Double Shake Co.
Dunau Associates
Dupont Forestry Products
Earth Justice Legal Defense Fund
East Lake WA Audubon Society
Eastern Washington University
Ebel & Associates
Elma Truck & TLR
Environmental Outlook, University of Washington
F.O.C.U.S.
Federal Lands Advisory Committee
Ferry County Action League
Forest Recovery-Granger Co.
Forest Stewards Guild, Northwest Regional Chapter
Forestry Sciences Lab
Fort James Corporation
Foster Wheeler Environmental Corp.
Friends of the Earth
Georgia Pacific Corp.
Grassland West Co.
Greater Greenwater Gateway Committee
Guy Bennett Lumber Co.
Inland Empire Paper Co.
Inland Empire Public Lands Council
James River Corp.
Kamerrer Family Farms
Kettle Range Conservation Group
The Lands Council
Lusignan Forestry, Inc.
Mentor Law Group
Methow Valley News
Methow Valley Snowmobile Association
Mountaineers
Mountains to Sound Greenway
National Audubon Society

National Audubon Society, Seattle Audubon Society
National Campers & Hikers
National Outdoor Leadership School, Pacific
National Wildlife Federation
Natural Resources Defense Council
The Nature Conservancy
North Cascade Audubon Society
North Cascades Conservation Council
North Cascades Institute
Northeast Washington Medical Group
Northwest Coalition for Alternatives to Pesticides
Northwest Ecosystem Alliance
Northwest Fly Anglers
Northwest Forestry Association
Northwest Timber Workers Resource Council
Okanogan Highlands Alliance
Okanogan Resource Council
Olympia Forest Sciences Laboratory
The Omak County Chronicle
P.L.U.S.
Pacific Biodiversity Institute
Pacific Crest Biodiversity Project
Pacific Rivers Council
Pilchuck Audubon Society
Plum Creek Timber Co.
Ponderay Newsprint Co.
Potlatch Corporation
Public Land Users Society
Raedeke Association., Inc.
Rafter Seven Ranch
Rainier Audubon Society
Resources Northwest, Inc.
Rivers Council of Washington
Rocky Mountain Elk Foundation
Rosboro Lumber Co.
RZ Resource Consultants
Seattle Audubon Society
Seattle Snohomish Mill Co.
Seattle University
Sierra Club
Sierra Club Legal Defense Fund
Sierra Club Northern Rockies Chapter
Sierra Club, Northwest Office

Signpost Magazine
Skagit Valley College
Skyline Wheat Ranch
Society of American Foresters
Spokane Research Center
Stevens Pass Ski Area
Tahoma Audubon Society
The Trust for Public Lands
Trout Unlimited, NW Steelhead & Salmon Council
Umatilla Forest Watch
University of Puget Sound
University of Washington
University of Washington, College of Forest Resources
Upper Columbia Resource Council
Vaagen Brothers Lumber Co.
Volunteers for Outdoor Washington
Washington Native Plant Society
Washington Contract Loggers Association
Washington Environmental Council
Washington Farm Forestry Association
Washington Forest Protection Association
Washington Friends of Farms & Forests
Washington Native Plant Society
Washington State Department of Ecology
Washington State Department of Fish & Wildlife
Washington State Farm Bureau
Washington State Mineral Council
Washington State Snowmobile Association
Washington State University
Washington Wilderness Coalition
Western Land Exchange Project
Western Resource Analysis
Western Washington University
Weyerhaeuser Co.
White River Recreation Association
Whitman College
Wild Washington Campaign
Wilderness Society
Wilderness Watch
Willapa Hills Audubon Society, Conservation B
Zahn Ranch

West Virginia

Allegheny Wood Products, Inc.
Alyeska, Inc.
Appalachian Investments
Appalachian Trail Conference
Bluefield State College
Central Tie & Lumber Co.
Coastal Lumber Co.
Concord College
Davis & Elkins College
Davis & Elkins College, Department of Biology
The Garden Works
Fairmont State College
Fairmont State College, Department of Biology
Hardscrabble Enterprises, Inc.
Harmony Hill
Lapaix Farm
Marshall University
Marshall University, Department of Biological Services
Millstone Farm
Monongahela Power Co.
Mountain Aquaculture & Producers Association
The Mulch Patch
New Dawn Farm
Parsons Volunteer Fire Department
Salem-Teikyo University
Shepherd College
Sierra Club, West Virginia Chapter
Sleepy Creek Seed Co.
Tilinghast & Neely
Twin Oaks Farm & Nursery
West Virginia Bass Federation
West Virgina Forestry Association
West Virgina RC&D Association
West Virgina Sierra Club
West Virginia State College
West Virginia University
West Virginia University CES
West Virginia University Division of Plant & Soil Sciences
Westvaco Corp.
Wheeling Jesuit University
Woodland Owners Association of West Viginia

Wisconsin

American Pulpwood Association
Beloit College
Blue Ox Forestry Service, Inc.
Boardman, Suhr, Curry & Field
Burns Forestry Consultants
Consolidated Papers, Inc.
Florence Mining News
Forestry Services Co
Fox Valley Technical College
Georgia Pacific
Johnson Timber Corp.
Lake States Forestry
Lake States Independent Loggers
Lake States Women in Timber
Lawrence University
Lodholz North Star Acres
Louisiana-Pacific Corporation
M&M Associates
Meier Natural Resources Conservation
Michigan-Wisconsin Timber Producers Association
National Association of Conservation District
Natural Resources Services & Consulting
Oakwood Forestry Consulting
Pierre & Sweeney Lawyers
Pine River Lumber Co.
Rhinelander Daily News
Ruffed Grouse Society
Sierra Club - Midwest Region
Steigerwaldt Land Services
Tappon-Ruetz Land Services, Inc.
Thilmany Paper Co.
Tigerton Lumber Co.
Triple "T" Enterprises, Inc.
University of Wisconsin, Green Bay College of Environmental Science
University of Wisconsin Extension
University of Wisconsin, Stevens Point School of Natural Resources
University of Wisconsin, Eau Claire
University of Wisconsin, Green Bay
University of Wisconsin, La Crosse
University of Wisconsin, Madison

Vilas County News Review
Wausau Paper Mills Co.
Whitetails Unlimited, Inc.
Wisconsin Audubon Society
Wisconsin County Forests Association
Wisconsin Forest Conservation Task
Wisconsin Forest Productivity Council
Wisconsin Paper Council
Wisconsin Woodland Owners Association

Wyoming

Central Wyoming College
Northern Rockies Conservation Cooperative
Northwest College
Sierra Club, Wyoming Chapter
University of Wyoming

Canada

Bioforest Tech., Inc.
Valent Biosciences
3M Canada Co Ltd

6.5 Libraries

Alabama

Alabama A&M University, J.F. Drake Memorial Library
Andalusia Public Library
Attalla-Etowah County Public Library
Auburn University at Montgomery Library
Baldwin County Library Cooperative
Cherokee County Public Library
Choctaw County Public Library
Clayton-Town & County Library
De Kalb County Public Library
Fayette County Memorial Library
Gadsden-Etowah County Library
Hale County Public Library
Huntsville-Madison County Public Library
Jacksonville State University, Houston Cole Library
Lawrence County Public Library

Macon County-Tuskegee Public Library
Marengo County Public Library
Marion-Perry County Library
Monroe County Public Library
North Shelby County Library
Phenix City-Russell County Library
Saint Clair County Library
Sumter County Library System
Troy State University, Library, Wallace Hall
Tuskegee University, Hollis Burke Frissell Library
University of Alabama, Amelia Gayle Gorgas Library
University of Alabama, Huntsville, Salmon Library
University of South Alabama, University Libraries
Washington County Public Library
Wilcox County Library

Alaska

Alaska Resources Library & Information Service
Anchorage Municipal Libraries
Arlis Library
University of Alaska, Anchorage Consortium Library
University of Alaska, Fairbanks, Elmer E. Rasmuson
     Library
University of Alaska, Southeast, Ketchikan Campus
     Library
University of Alaska, Southeast, William A. Egan
     Library
Z.J. Loussac Public Library

Arizona

Arizona State Library,
Clifton-Greenlee County Public Library
Cochise County Library District
Flagstaff City-Coconino County Public Library
Gila County Library District
Huachuca City Public Library
Maricopa County Library District
Mohave County Library District
Northern Arizona University Cline Library
Phoenix Public Library
University of Arizona Main Library

Yavapai County Library District
Yuma County Library District
Yuma County Library District Main Library

Arkansas

Arkansas State Library
Arkansas State University, Jonesboro, Dean B. Ellis
     Library
Central Arkansas Library System Main Library
Cleburne County Library
Conway County Library Headquarters
Craighead County & Jonesboro Public Library
Crittenden County Library
Dallas County Library
Faulkner County Library
Garland County Library
Grant County Library
Hot Spring County Library
Izard County Library-Melbourne
Jackson County Library
Lawrence County Library
Lonoke Prairie County Regional Library
Lyon College Mabee-Simpson Library
Montgomery County Library
Ouachita Baptist University Riley-Hickingbotham
     Library
Pine Bluff & Jefferson County Library System
Poinsett County Library
Pope County Library System
Saline County Public Library
Southern Arkansas University Magale Library
University of Arkansas, Mullins Library
University of Arkansas - Little Rock, Ottenheimer
     Library
University of Arkansas, Monticello Library
University of Arkansas - Pine Bluff, Watson Memorial
     Library
University of Central Arkansas Torreyson Library
University of the Ozarks Robson Library

California

Alameda County Library
Alpine County Free Library
Anaheim Public Library
Berkeley Public Library
Butte County Library
California Institute of Technology Library
California Polytechnic State University Library
California State Library
California State University, Bakersfield Library
California State University, Chico Library
California State University, Dominguez Hills Library
California State University, Fresno Library
California State University, Fullerton Library
California State University, Hayward Library
California State University, Long Beach Library
California State University, Los Angeles Library
California State University, Northridge Library
California State University, San Bernardino Library
California State University, San Marcos Library
California State University, Stanislaus Library
Carlsbad City Library
Claremont University Center Library
Colusa County Free Library
Contra Costa County Library
College of the Sequoias Library
County of Los Angeles Public Library
Daly City Public Library
Del Norte County Library District
Fresno County Free Library
Fresno County Genealogical Society Library
Fresno County Public Library
Humboldt County Library
Humboldt State University Library
Kern County Library
Kern County Library System Library
Lake County Library
Los Angeles Public Library System
Los Gatos Public Library
Madera County Library
Marin County Free Library
Mariposa County Library
Mendocino County Library

Merced County Library
Modoc County Library
Mono County Free Library System-Northern Region
Mono County Free Library System-Southern Region
Monterey County Free Libraries
Nevada County Library
Oakland Public Library
Orange County Public Library
Pepperdine University Library
Plumas County Library
Riverside Public Library
Sacramento Public Library
San Benito County Free Library
San Bernardino County Library
San Diego County Library
San Diego Public Library Library
San Diego State University Library
San Francisco State University Library
San Jose State University Library
San Leandro Public Library
San Luis Obispo City-County Library
San Mateo County Library
Santa Clara County Free Library
Santa Clara University Library
Santa Cruz City-County Library System Headqua
Shasta County Library
Solano County Library System Library
Sonoma County Public Library
South San Francisco Public Library
Stanford University Library
Stanislaus County Free Library
Sutter County Free Library
Thousand Oaks Library
Tulare County Library System
Tuolumne County Free Library
University of California, Berkeley Library
University of California, Berkeley School of Law
    Library
University of California, Davis Library
University of California, Irvine Library
University of California, Los Angeles Library
University of California, Riverside Library
University of California, San Diego Library

University of California, Santa Barbara Library
University of California, Santa Cruz Library
University of Redlands Library
University of San Francisco Library
University of Southern California Library
Ventura County Library Services Agency
Whittier College Library
Yolo County Library
Yuba County Library

Colorado

Adams State College Library
Arkansas Valley Regional Library Service System
Baca County Public Library
Colorado College Library
Colorado School of Mines Library
Colorado State Library
Colorado State University Library
Conejos County Library
Delta County Public Library
Denver Public Library
Dolores County School Public Library
East Morgan County Library District
Elbert County Library
Garfield County Public Library System
Gilpin County Public Library
Grand County Library District
Gunnison County Public Library
Jackson County Public Library
Jefferson County Public Library
Kiowa County Public Library
Lake County Public Library
Las Animas-Bent County Public Library
Mesa County Public Library District
Mesa County Public Library District
Mesa State College Library
Mineral County Regional Library
Moffat County Libraries
Park County Public Library
Pitkin County Library
Pueblo Library District
Regis University Library
San Miguel County Public Library District No

Southern Peaks Public Library
University of Colorado, Boulder Library
University of Colorado, Colorado Springs Library
University of Colorado, Denver Library
University of Denver Library
University of Northern Colorado Library
University of Southern Colorado Library

Connecticut

Central Connecticut State University Library
Connecticut College Library
Connecticut State Library
Danbury Public Library
Eastern Connecticut State University Library
Hartford Public Library
Quinnipiac University Library
Southern Connecticut State University Library
Teikyo Post University Library
Trinity College Library
Union Free Public Library
University of Connecticut Library
University of New Haven Library
Wesleyan University Library
Western Connecticut State University Library
Yale University Library

Delaware

Appoquinimink Community Library
Delaware State University Library
Delaware Technical & Community College Library
New Castle County Public Library System
Sussex County Department of Libraries
University of Delaware Library

District of Columbia

American University Library
Catholic University of America Library
District of Columbia Public Library
Georgetown University Library
Library of Congress Library
U.S. Department of the Interior Departmental Library
U.S. Senate Library

Florida

Alachua County Library District Headquarters
Bradford County Public Library
Brevard County Library System
Broward County Division of Libraries
Calhoun County Public Library
Collier County Public Library
Columbia County Public Library
Flagler County Public Library
Florida Atlantic University Library
Florida Institute of Technology Library
Franklin County Public Library
Hardee County Public Library
Hendry County Library System
Hernando County Public Library System
Highlands County Library System
Holmes County Library
Indian River County Main Library
Jacksonville Public Library
Jacksonville University Library
Jefferson County Public Library
Lake County Library System
Lee County Library System
Manatee County Public Library System
Martin County Library System
Monroe County Public Library
North Indian River County Library
Osceola County Library System
Palm Beach County Genealogical Society Librar
Palm Beach County Library System
Pasco County Library System
Putnam County Library System
Saint Johns County Public Library System
Saint Lucie County Library System
Seminole County Public Library System
State Library of Florida
Stetson University Library
Tampa-Hillsborough County Public Library
Taylor County Public Library
Union County Public Library
University of Florida Library
University of Miami Library
University of North Florida Library

Volusia County Public Library
Wakulla County Public Library
Washington County Library

Georgia

Athens Clarke County Library
Atlanta-Fulton Public Library
Bartow County Library System
Brooks County Public Library
Brunswick-Glynn County Regional Library
Chattooga County Library
Clayton County Library System
Coastal Plain Regional Library
Cobb County Public Library System
Dekalb County Public Library
Dougherty County Public Library
Elbert County Library
Fitzgerald-Ben Hill County Library
Gwinnett County Public Library
Hart County Library
Houston County Public Library System
Jefferson County Library System
Newton County Library
Thomas County Public Library System
Troup-Harris-Coweta Regional Library

Hawaii

Hawaii State Library
Hawaii State Library System
Hawaii State Public Library System

Idaho

American Falls District Library
Boundary County District Library
Camas County Public Library
Cambridge Community Library
Clark County District Library
East Owyhee County Library District
Idaho Falls Public Library
Idaho State Library
Idaho State Talking Book Library
Jefferson County District Library

Latah County Free Library District
Menan County District Library
Midvale District Library
Oneida County Free Library

Illinois

Brown County Public Library District
Calumet City Public Library
Champaign Public Library
Decatur Public Library
Evansville Public Library
Henderson County District Library
La Grange Park Public Library District
Northern Illinois Library System
Putnam County Public Library District
South County Public Library District
Warren County Public Library District

Indiana

Allen County Public Library
Bartholomew County Public Library
Benton County Public Library
Crawford County Public Library
Fayette County Public Library
Fulton County Public Library
Greensburg-Decatur County Public Library
Indianapolis-Marion County Public Library
Jackson County Public Library
Jasper County Public Library
Jay County Public Library
Monroe County Public Library
Morgan County Public Library
Newton County Public Library
North Madison County Public Library System
Ohio County Public Library
Owen County Public Library
Pike County Public Library
Saint Joseph County Public Library
Scott County Public Library
Spencer County Public Library
Sullivan County Public Library
Switzerland County Public Library

Tell City-Perry County Public Library
Tippecanoe County Public Library
Tipton County Public Library
Union County Public Library

Iowa

Altoona Public Library
Ames Public Library
Cumberland Public Library
Dubuque County Library
Public Library of Des Moines
Scott County Library System
State Library of Iowa
Union Public Library
Woodbury County Rural Library

Kansas

Coffey County Library
Finney County Public Library
Graham County Public Library
Grant County Library
Hamilton County Library
Johnson County Library
Kearny County Library
Kiowa County Library
Linn County Library District Three
Linn County Library District Two
Linn County Library District One
Morton County Library
Scott County Library
Sheridan County Library
Stanton County Library
Stevens County Library
Wichita County Library

Kentucky

Allen County Public Library
Boone County Public Library
Bowling Green Public Library
Boyd County Public Library
Boyle County Public Library
Breathitt County Library

Breckinridge County Public Library
Calloway County Public Library
Campbell County Public Library
Carroll County Public Library
Casey County Public Library
Clark County Public Library
Clinton County Public Library
Crittenden County Public Library
Cumberland County Public Library
Cynthiana-Harrison County Public Library
Daviess County Public Library
Edmonson County Public Library
Estill County Public Library
Fleming County Public Library
Floyd County Public Library
Gallatin County Public Library
Garrard County Public Library
Grant County Public Library District
Graves County Library
Grayson County Public Library
Hancock County Library
Hardin County Public Library
Harlan County Public Library
Hart County Public Library
Henderson County Public Library
Henry County Library
Hopkins County-Madisonville Public Library
Jackson County Public Library
Johnson County Public Library
Kenton County Public Library
Kentucky Department for Libraries
Knott County Public Library
Knox County Public Library
Laurel County Public Library District
Lawrence County Public Library
Lee County Public Library
Leslie County Library
Lewis County Public Library
Logan County Public Library
Louisville Free Public Library
Lyon County Public Library
Madison County Public Library
Magoffin County Library

Marion County Public Library
Marshall County Public Library
Mason County Public Library
McCreary County Public Library District
Meade County Public Library
Menifee County Public Library
Mercer County Public Library
Metcalfe County Public Library
Middlesboro-Bell County Public Library
Monroe County Public Library
Nicholas County Public Library
Ohio County Public Library
Oldham County Public Library
Owen County Public Library
Pendleton County Public Library
Pike County Public Library District
Powell County Public Library
Pulaski County Public Library
Robertson County Public Library
Rockcastle County Library
Rowan County Public Library
Russell County Public Library
Scott County Public Library
Spencer County Public Library
Taylor County Public Library
Todd County Public Library
Trimble County Public Library
Union County District Library
Washington County Public Library
Wayne County Public Library
Whitley County Library
Wolfe County Library

Louisiana

Beauregard Parish Public Library
Bossier Central Library
Caldwell Parish Library
Catahoula Parish Library
Claiborne Parish Library
Concordia Parish Library
Jefferson Parish Library Department
Lafourche Parish Public Library
Saint Martin Parish Library

Saint Mary Parish Library
Southern University, New Orleans
State Library of Louisiana
Tangipahoa Parish Library
Union Parish Library
Washington Parish Library System
West Carroll Parish Library
Winn Parish Library

Maine

Maine State Library
Portland Public Library

Maryland

Baltimore County Public Library
Calvert County Public Library
Caroline County Public Library
Carroll County Public Library
Dorchester County Public Library
Frederick County Public Libraries
Harford County Public Library
Kent County Public Library
Montgomery County Department of Public Library
Prince George's County Memorial Library System
Queen Anne's County Free Library
Saint Mary's County Memorial Library
Somerset County Library System
Talbot County Free Library
Washington County Free Library
Worcester County Library

Massachusetts

Boston Public Library
Cambridge Public Library
State Library of Massachusetts
Worcester Public Library

Michigan

Alcona County Library System
Alpena County Library
Ann Arbor District Library
Bay County Library System

Benton Harbor Public Library
Charlevoix Public Library
Crawford County Library
Detroit Public Library
Dickinson County Library
East Lansing Public Library
Flint Public Library
Gladwin County Library
Jackson District Library
Kalkaska County Library
Lapeer County Library
Macomb County Library
Manistee County Library
Mason County District Library
Menominee County Library
Mideastern Michigan Library Cooperative
Missaukee District Library
Monroe County Library System
Muskegon County Library
Oakland County Library
Otsego County Library
Petoskey Public Library
Shiawassee County Library
St. Clair County Library
Washtenaw County Library

Minnesota

Anoka County Library
Anoka County Library System
Carver County Library
Douglas County Library
Fergus Falls Public Library
Great River Regional Library
Hennepin County Library
Hennepin County Library System
Jackson County Library System
Marshall-Lyon County Library
Martin County Library
Milaca Community Library
Montevideo-Chippewa County Public Library
Nobles County Library & Information Center
Ramsey County Public Library
Rock County Community Library

Saint Paul Public Library
Scott County Library System
Washington County Library
Watonwan County Library

Mississippi

Biloxi Public Library
Hancock County Library System
Harrison County Library System
Humphreys County Library System
Laurel-Jones County Library
Madison County-Canton Public Library
Marks-Quitman County Library
Marshall County Library System
Mississippi Library Commission
Neshoba County Public Library
Noxubee County Library System
Raleigh Public Library'
Sunflower County Library System
Tallahatchie County Library
Union County Library
Warren County-Vicksburg Public Library
Washington County Library System

Missouri

Bollinger County Library
Camden County Library District
Cass County Public Library
Christian County Library
Dallas County Library
Daviess County Library
Douglas County Public Library
Gentry County Library
Grundy County-Jewett Norris Library
Henry County Library
Jefferson County Library
Kansas City Public Library
Livingston County Library
Mcdonald County Library
Mercer County Library
Miller County Library Service Center
Mississippi County Library

Missouri State Library
Neosho Newton County Library
New Madrid County Library
Ozark County Library
Putnam County Public Library
Ralls County Library
Ray County Library
Saint Charles City County Library District
Saint Clair County Library
Saint Louis County Library
Scotland County Memorial Library
Springfield-Greene County Library
Stone County Library
Sullivan County Public Library
Washington County Library
Wright County Library

Montana

Blaine County Library
Chouteau County Library
Daniels County Free Library
Flathead County Library
Garfield County Library
Glacier County Library
Glasgow City County Library
Liberty County Library
Lincoln County Public Libraries
Livingston-Park County Library
Meagher County City Library
Mineral County Public Library
Missoula Public Library
Montana State Library
Petroleum County Community Library
Prairie County Library
Roosevelt County Library
Rosebud County Library
Sheridan County Library
Stillwater County Library
Thompson-Hickman Free County Library

Nebraska

Garfield County Library
Grant County Library
Hooker County Library
Lincoln City Libraries
Logan County Library
Omaha Public Library
Rock County Public Library
South Sioux City Public Library
Thomas County Library

Nevada

Carson City Library
Douglas County Public Library
Elko-Lander-Eureka County Library System
Humboldt County Library
Las Vegas-Clark County Library District
Lincoln County Library
Lyon County Library System
Mineral County Public Library
Pershing County Library
Storey County Public Library
Washoe County Library
White Pine County Library

New Hampshire

East Rochester Public Library
Nashua Public Library
Unity Free Public Library

New Jersey

Atlantic City Free Public Library
Atlantic County Library
Burlington County Library
Camden County Library System
Cape May County Library
Cumberland County Library
Gloucester County Library System
Hunterdon County Library
Mercer County Library
Monmouth County Library
Morris County Library

Somerset County Library
Sussex County Library System
Trenton Public Library
Warren County Library

New York

Albany Public Library
Brooklyn Public Library
Broome County Public Library
East Rochester Public Library
La Grange Association Library
Monroe County Library System
Mount Vernon Public Library
New York Public Library
Onondaga County Public Library
Queens Borough Public Library
Schenectady County Public Library
South Country Library
South New Berlin Free Library
Tompkins County Public Library

North Carolina

Alexander County Library
Asheville-Buncombe Library System
Avery County Morrison Public Library
Bladen County Public Library
Brunswick County Library
Burke County Public Library
Caldwell County Public Library
Carteret County Public Library
Catawba County Library
Columbus County Public Library
Cumberland County Public Library & Informatio
Currituck County Public Library
Dare County Library
Davidson County Public Library System
Davie County Public Library
Duplin County Library
Durham County Library
Edgecombe County Memorial Library
Forsyth County Public Library
Franklin County Library

Gates County Library
Graham County Public Library
Granville County Library System
Greene County Public Library
Harnett County Public Library
Havelock-Craven County Library
Haywood County Public Library
Henderson County Public Library
Hertford County Library
Hoke County Public Library
Iredell County Public Library
Jackson County Public Library
Kinston-Lenoir County Public Library
Lee County Library
Lincoln County Public Library
Macon County Public Library
Madison County Public Library
Mcdowell County Public Library
Mitchell County Public Library
Montgomery County Public Library
New Hanover County Public Library
Onslow County Public Library
Pamlico County Library
Pender County Library
Perquimans County Library
Person County Public Library
Robeson County Public Library
Rockingham County Public Library
Scotland County Memorial Library
Stanly County Public Library
State Library of North Carolina
Transylvania County Library
Tyrrell County Public Library
Union County Public Library
Warren County Memorial Library
Washington County Library
Watauga County Public Library
Wayne County Public Library, Inc.
Wilson County Public Library
Yancey County Public Library

North Dakota

Bottineau County Public Library
Cavalier County Library
Kidder County Library
Mckenzie County Public Library
Morton County Library
Stutsman County Library
Valley City-Barnes County Public Library
Ward County Public Library

Ohio

Adams County Public Libraries
Akron-Summit County Public Library
Brown County Public Library
Carroll County District Library
Champaign County Library
Clark County Public Library
Clermont County Public Library
County District Library
Cuyahoga County Public Library
Delaware County District Library
Findlay-Hancock County Public Library
Geauga County Library System
Geauga County Public Library
Greene County Public Library
Guernsey County District Public Library
Highland County District Library
Logan County District Library
Medina County District Library
Meigs County District Public Library
Mercer County District Library
Monroe County District Library
Paulding County Carnegie Library
Perry County District Library
Pickaway County District Public Library
Pickerington Public Library
Portage County District Library
Preble County District Library
Public Library of Cincinnati
Stark County District Library
State Library of Ohio
Troy-Miami County Public Library

Tuscarawas County Public Library
Warren-Trumbull County Public Library
Washington County Public Library
Wayne County Public Library
Williams County Public Library

Oklahoma

Beaver County Pioneer Library
Cherokee-City-County Public Library
Choctaw County Library
Delaware County Library
Latimer County Public Library
Metropolitan Library System
Nowata City-County Library
Tulsa City-County Library
Woodward Public Library

Oregon

Baker County Public Library
Clackamas County Library
Corvallis-Benton County Public Library
Crook County Library
Deschutes County Library System
Douglas County Library System
Gilliam County Library
Grant County Library
Harney County Library
Hood River County Library
Jackson County Library Services
Jefferson County Library
Josephine County Library System
Klamath County Library
La Grande Public Library
Malheur County Library
Multnomah County Library
The Dalles-Wasco County Library
Tillamook County Library
University of Oregon Library
Washington County Cooperative Library Service

Pennsylvania

Adams County Library System
Allegheny County Library
Altoona Area Public Library
Bedford County Library
Blair County Library System
Bradford County Library System Headquarters
Bucks County Free Library
California University of Pennsylvania Louis L.
    Manderino Library
Cambria County Library System & District Cent
Cameron County Public Library
Carnegie Library of Pittsburgh
Centre County Library & Historical Museum
Chester County Library
Clearfield County Public Library Federation
Columbia County Traveling Library
Crawford County Federated Library System
Dauphin County Library System
Delaware County Library System
Dickinson College Waidner-Spahr Library
Dormont Public Library
Erie County Public Library
Fayette County Library System
Forest County Library
Franklin County Library System
Free Library of Philadelphia
Fulton County Library
Greene County Library System
Huntingdon County Library
Juniata County Library, Inc.
Lackawanna County Library System
Lancaster County Library
Lawrence County Law Library
Lebanon County Library System
Mifflin County Library
Montgomery County-Norristown Public Library
Perry County Law Library
Pike County Public Library
Snyder County Library
Somerset County Library
State Library of Pennsylvania
Sullivan County Library

Wayne County Public Library
West Chester Public Library
York County Library System

Rhode Island

Rhode Island State Library

South Carolina

Beaufort County Library
Berkeley County Library
Calhoun County Library
Cherokee County Public Library
Chester County Library
Chesterfield County Library
Colleton County Memorial Library
Darlington County Library
Dillon County Library
Dorchester County Library
Fairfield County Library
Florence County Library
Georgetown County Library System
Greenville County Library
Harvin Clarendon County Library
Horry County Memorial Library
Kershaw County Library
Lancaster County Library
Laurens County Library
Lee County Public Library
Marion County Library
Oconee County Library
Orangeburg County Library
Pickens County Library System
Richland County Public Library
Saluda County Library
Spartanburg County Public Libraries
Spartanburg County Public Library
Sumter County Library
Union County Carnegie Library
Williamsburg County Library
York County Library

South Dakota

Bennett County Public Library
Custer County Library
Grant County Public Library
Hand County Library
Hyde County Library
Jackson County Library
Potter County Free Public Library
Sully County Library
Tripp County Library-Grossenburg Memorial

Tennessee

Benton County Library
Bledsoe County Public Library
Blount County Public Library
Bolivar-Hardeman County Public Library
Carroll County Library
Chattanooga-Hamilton County Bicentennial Library
Cheatham County Public Library
Chester County Public Library
Claiborne County Public Library
Coffee County Lannom Memorial Public Library
Coffee County-Manchester Library
Decatur County Library
Decatur-Meigs County Library
Dickson County Public Library
Fayetteville-Lincoln County Public Library
Fentress County Public Library
Franklin County Library
Gibson County Memorial Library
Giles County Public Library
Hancock County Public Library
Hardin County Library
Houston County Public Library
Humphreys County Public Library
Johnson County Public Library
Knox County Public Library System
Lawrence County Public Library
Lebanon-Wilson County Library
Lewis County Public Library
Macon County Public Library
Marshall County Memorial Library

Maury County Public Library
Memphis-Shelby County Public Library
Moore County Public Library
Mount Juliet-Wilson County Public
Overton County Public Library
Perry County Public Library
Pickett County Library
Public Library of Nashville & Davidson
Putnam County Library System
Rutherford County Library System
Scott County Public Library
Sequatchie County Public Library
Sevier County Public Library
Smith County Public Library
Stewart County Public Library
Sullivan County Public Library
Tipton County Public Library
Unicoi County Public Library
Washington County - Jonesborough Library
Wayne County Public Library
Williamson County Public Library

Texas

Alamo Area Library System
Amarillo Public Library
Arlington Public Library
Bandera County Library
Bee County Public Library
Brazoria County Library System
Brooks County Library
Calhoun County Library
Callahan County Library
Carson County Public Library
Central Texas Library System
Chambers County Library System
Coke County Library
Cooke County Library
Corsicana Public Library
Crane County Library
Crockett County Public Library
Crosby County Library
Dallas Public Library
Dawson County Library

Delta County Public Library
Dickens County-Spur Public Library
Dimmit County Public Library
East Parker County Library
Ector County Library
Floyd County Library
Ford County Library
Fort Bend County Libraries
Gaines County Library
Goliad County Library
Hansford County Library
Harris County Public Library
Haskell County Library
Hemphill County Library
Hidalgo County Library System
Hockley County Memorial Library
Hood County Public Library
Houston Public Library
Huntsville Public Library
Irion County Library
Jackson County Library
Jefferson County Library
Karnes County Library System
Kaufman County Library
Kent County Library
Kimble County Library
Kinney County Public Library
Lamb County Library
Leon Valley Public Library
Live Oak County Library
Llano County Library System
Madison County Library
Martin County Library
Memphis Public Library
Mitchell County Public Library
Montgomery County Memorial Library System
Newton County Public Library
North Texas Regional Library System
Oldham County Library
Rains County Public Library
Reagan County Library
Real County Public Library
Red River County Public Library

Reeves County Library
Roberts County Library
Rockwall County Library
San Patricio County Library System
San Saba County Library
Schleicher County Public Library
Scurry County Library
Shackelford County Library
Sherman County Public Library
Somervell County Library
Starr County Public Library
Stonewall County Library
Sutton County Library
Sweetwater County-City Library
Swisher County Library
Terrell County Public Library
Texas State Law Library
Upshur County Library
Upton County Public Library
Val Verde County Library
Waco-Mclennan County Library
Waller County Library
Wilson County Library
Winkler County Library
Yoakum County Library
Yoakum County Library
Zapata County Public Library

Utah

Davis County Library
Grand County Public Library
Morgan County Library
Salt Lake County Library System
San Juan County Library
Uintah County Library
Utah State University Natural Resources Research
    Library
Wasatch County Library
Washington County Library
Weber County Library

Vermont

Aldrich Public Library
Bennington Free Library
Landgrove Public Library
Orwell Free Library
State of Vermont Department of Libraries
Thetford Town Library

Virginia

Appomattox Regional Library
Arlington County Department of Libraries
Botetourt County Library
Campbell County Public Library
Charlotte County Library
Chesterfield County Public Library
Colonial Heights Public Library
County of Henrico Public Library
Culpeper County Library
Cumberland County Public Library
Fauquier County Public Library
Fluvanna County Library
Franklin County Library
Jefferson-Madison Regional Library
Loudoun County Public Library
Norfolk Public Library System
Nottoway County Library
Orange County Library
Pittsylvania County Public Library
Powhatan County Public Library
Pulaski County Library
Rappahannock County Library
Roanoke County Public Library
Russell County Public Library
Shenandoah County Library
Tazewell County Public Library
Washington County Public Library
York County Public Library

Washington

Asotin County Library
Forest Resource Library
King County Library System

Pacific Marine Technology Library
Pierce County Library System
Spokane County Library District
Spokane Public Library
Stevens County Rural Library District
Tacoma Public Library
Walla Walla County Library
Washington State Law Library
Washington State Library
Whitman County Rural Library

West Virginia

Alpha Regional Library
Brooke County Public Library
Cabell County Public Library
Calhoun County Public Library
Clarksburg-Harrison Public Library
Doddridge County Public Library
Elkins-Randolph County Public Library
Fayette County Public Libraries
Five Rivers Public Library
Grant County Library
Greenbrier County Library
Hamlin-Lincoln County Public Library
Hampshire County Public Library
Hardy County Public Library
Jackson County Library
Kanawha County Public Library
Keyser-Mineral County Public Library
Marion County Public Library
Mason County Library System
Mingo County Library
Monroe County Public Library
Morgan County Public Library
Ohio County Public Library
Pendleton County Public Library
Pleasants County Public Library
Pocahontas County Free Library
Putnam County Library
Raleigh County Public Library
Ritchie County Public Library
Roane County Public Library
Summers County Public Library

Taylor County Public Library
Tyler County Public Library
Upshur County Public Library
West Virginia University Evansdale Library
Wyoming County Library System

Wisconsin

Cumberland Public Library
Dodge County Library Service
Door County Libraries
Florence County Library
Marathon County Public Library
Marinette County Consolidated Public Library
Menominee Tribal County Library
Milwaukee County Federated Library System
Oneida County Mailbox Library
Portage County Public Library
Western Taylor County Public Library
Wisconsin State Law Library

Wyoming

Campbell County Public Library
Carbon County Library System
Converse County Library
Crook County Library
Goshen County Library
Hot Springs County Library
Johnson County Library
Laramie County Library System
Lincoln County Library
Natrona County Public Library
Niobrara County Library
Park County Library System
Platte County Public Library
Sheridan County Fulmer Public Library
Sublette County Library
Sweetwater County Library System
Teton County Library
Uinta County Library
Western Wyoming Community College Library

6.6 Individuals

Alabama

Timothy Boyce
 J. Wayne Brewer
Jim Hyland
Adam Jackson
Tomm Johnson
Bill Moore
M. N. "Corky" Pugh
Bruce Shupp
Debi Summersell

Alaska

Randy Bates
Candace Beery
Charles Bell
Joel Bennett
Tim Blust
Corrie Bosman
Roger Burnside
Andre Camara
Niles Cesar
Melanie Duchin
Hugh Durham IV
Ken Fisher
John Fox
Sylvia Geraghty
Bob Gorman
Owen Graham
Mike Holloway
Eric Hummel
Jeff Jahnke
Edgar Jenks
Christina Jewett
Jason Loos
Gary Morrison
Don Muller
Mary Pete
Frank Rue
Joseph Sebastian
Marlo Shedlock

Fred Sorenson
Dave Sturdevant
Tara Sweeney
Doug Warner
Ronald Wolfe

Arizona

Thomas Abrams
Gale & Vesta Aldrich
Peggy Alexander
Theresa L. Allison
Andrea Anderson
Fred Anderson
Kathryn Anderson
John Anhold
Scott and Toni Arena
Donald Arganbright
Don Arkin
Rachel Aschmann
Janina Austin
Jean & Trevor Avenett
Charles Babbitt
Pat Baca, Jr.
Paula Bachman-Williams
John Baker
Theodore Barbone
Ariel Barfield
Annie Barva
Beverly Bass
Charles & Mar Bast
Kay Bawden
Don Beck
Paul Beier
Cher Beilfuss
William Beloret
Fray H. Belshe
Robert Bennet
Patsy Bennett
Linda Bentley
Don & Linda Bentley
David Bertelsen
Rebecca Berton
Andy Bessler

Carl Beyerhelm
Jessie Bhangoo
Bettina Bickel
Dennis J. Bigelow
Robert Bigelow
Rulon & Lucie Bigelow
Evelyn Billo
Toni Bish
Michael Bissontz
Joe Blaszczak
Edward Blumer
W. Brent & Joann Bogdanski
Larry Borden
Roy Boss
Melville S. Bowers
Virginia Gail Bowers
Curtis Bradley
Richard Bradshaw
Christine & M. Brady
Susan Brandes
Clait Braun
Ruth Brawdy
Robert Breen
Sheri Brennan
Ronald Brill
Scott Brill
ArthurandAnne Britt
Bob Broscheid
Grant & Innis Brown
Jarrod Brown
Kelli Brown
Rayanne & Jam Brown
Tonda Brown
James Brown
James A. Bruder
Adam Burdick
Jeff Burgess
Brian & Sarah M. Burnett
Carolyn Burns
M. & Jackie Butler
Paul Byers
John Caid
William Calder

Jean Calhoun
A. P. Camps
Stephen Canning
Lawrence Carlson
Larry Carlson
Royce Carlson
Ron Carswell
L. M. Case
Eligio & Anna Castillo
Mr. Bob Celaya
Don Chaney
Frank Chapman
Holden Chase
Alan Chatfield
Helen & Placi Chavez
Donna Chesner
Gary Christensen
Earl Christian
Paula Chronister
Ray & Sabine Cichlar
Becki Cimadevilla
Lamar & Pat Clark
Letha Cline
Christopher Cloud
Vonna Cluff
Joanne Cockerill
Mark Colby
Rose Coleman
A. Consolo
Mel Copeland
Brenda Corkin
Tania Corliss
Walter & R. Craig
Cullen Cramer
Cole Crocker-Bedford
Lauerl Crosby
Martha Crosby
Laura Cummings
Diane Cusack
Rudy Dankwort
Diana Davis
Vada L. Davis
Loren Dawn

Porter Dean
Roy & Joanne Dechant
Vic DeFrancesco
Tom DeGomez
Marc Delany
Eileen DeLauer
Shanna & Rod Denault
Charlie Denton
Sonya Diehn
Carl Dietrich
Linda Dills
Robert Dink, Jr.
John & Annie Dunn
Michael Durgain
Dane & Marlen Dyrland
Auroroa B. Eagar
Dustan & Cynthia Eagar
Roland & Ruth Eagar
Karen Earley
Barbara Edwards
David E. Eisley
Dan Ellerbroek
Dillie A. Ellis
Dock Ellis
Glen L. Ellison
Terrance Ely
Larry & Suzan Ely
Lloyd Engel
Rick Erman
Corwin Estes
Paula Fan
Christine Farney
Albert Farr
Dewey H. Farr
W. John Faust
James & Glend Finch
R. J. Finch
Brent Finley
Holly Finstrom
Heather Fitar
Bobby & Linda Fite
Chase & Lance Fite
M. Fitzgerald

Jenny Flynn	Scott Graff	David Hodges
Chris Ford	L. Graham	Bobbie Holaday
Tyler Forman	Richard Grapp	William Holden
B. & Kendie Foster	Jesse Greenberg	Jim & Karen Horton
Cheryl Foster	Michael Gregory	Caroline Hotaling
Zee Fowler	D. Grhoton	Michael Houghtaling
Linda Franks	Tim Griffith	Sally L. Hulsey
Steve Franks	Anastacia Gutierrez	Michael Humphrey
Dirk Frauenfelder	Brent Hall	Michael & Pat Humphrey
Rae Frederickson	Douglas & Michelle Hamblin	Kip & Alicia Hunsaker
Jim Frich	Lana Hamblin	Sean Hunsaker
Bruce Friedemann	Marion Hamblin	T. & A. Hunsaker
Kevin J. Fritz	Cheles Hancock	Joseph & Mari Hunter
Eric Gabel	Holly Hancock	Peter Ianchiou
Sharon Galbreath	Tina Hancock	Mike Ingram
Charles Gallagher	Robbie Hannawacker	Wayne D. Iverson
Victor Gallegos	Byron & Roz Harding	Glen Jacobs
Joe Ganey	Mr. & Mrs. Jeff Harper	David Jason Jaramillo
Karen R. Garley	N. Harper	Manuel Jaramillo
Ted Gartner	Ryan Harper	Jeff Jenness
Connie Gartner	Shae Lynn Harris	Shane & Paula Johnson
Robert Gay	Leo Hartke	Bonnie Johnson
Roxanne George	Syble & LaMar Hartley	Richard & Fran Jones
Roxane George	Meredith Hartwell	Mitzi Jones
Don Gerrard	Cynthia Hartzell	T. J. Jordan
Mary Ann Gibbons	Ron Harvey	Oweta Josleyn
Lee Roy Gibson	Michael Haseltine	Cecelia Juszczak
M. Gice	C. A. & Wilma Haught	Michelle Kaczynski
Curtis & Jean Gillespie	Jim & Sherri Haught	Charlie Kane
Brandon Gilliam	Kay Hauser	Joseph Kantauskis
D. Gilliam	Mike & Joelle Hauser	Jennifer Katcher
Joyce & Stone Gilliam	Steven & Christine Heap	John Keane
Robert & Elvia Gillies	Craig Hegel	Dennis Kee
Pamela Goldman	Merrill Hentz	Bart Keehn
Kenn Goldman	Robert Herdliska	Charlotte Keller
Robert Gomez	Terry Heslin	Patricia Kelly
Jerry Gonzales	Tom Hicks	M. Keoppen
Jose Gonzalez	Cody Hill	June A. & Don W. Kimble
Joni Goode	Ron Hill	Barbara & W. Kinman
Donald & Evel Goodman	Sky Hilts	Edward Kirsten
John & Karen Goodwin	Dawn Hines	Larry Kivela
Kenneth Gouker	Sidney Hirsh	Henry J. Klassen
Penny Govedich	Orne H. Hiscox	Keith Kleber

Barbara & Fred Klug

Edwin Knochel

Julie Kornmeyer

Kimberly Kuehnert

Paul Kuenstler

Gene & Barbar Laird

Jason Laird

Pierre Landau

Bob Landis

Linda Laney

G. James Langello

Kimberly Larsen

Alicia Larson

Susan Lascelles

Calvin Lash, Jr.

Arthur N. Lee

Danny Lee

Katherine Lee

Dan Leeds

Kent LeSueur

N. LeSueur

Vera LeSueur

Robin Levenworth

Lainie Levick

Jerry Levitt

Glen Lewis

H. Lewis

Timothy Lewis

Tracy Liston

Roy Little

Jose Logan

Manuel Logan

Duane Lowell

Karen Lowery

Sam A. Luce

Dave Lugers

Bradley Lundahl

Ann Lynch

Dan Lytle

Dave Madison

Joe Madrid

Robert Majors

Katie Malone

Pat & Sandra Malone

Lynn Marcus

Rich Marimow

Georgia E. Marks

Ginger Marks

Randy Marlatt

Rob Marshall

Fred Martin

Nita Martin

Stephanie Martin

Gerald & Sandy Martinez

Robert Mathieu

Perri Matthews

Donald & Susan Maxwell

Marlin Maxwell

Edward McCain

Bette McCall

Martha McClain

Max McClain

Lou McDonald

Kevbin McHugh

Jane McIntyre

Robert McIntyre

Richard McKee

Dorthy McKenna

Taylor McKinnon

Tamara McWhinney

Gary Mead

Kim Medina

Chris Mehling

Harry Melts

Bob & Bunny Meredith

Phillip Merkle

Karen Merrill

Lula Merrell

L. Vista Michael

Karen Midkiff

Len Milich

Dave Miller

Norma Miller

Lydia Millet

John Miranda

Leo Mobley

Ron Mohney

Rick Mohr

Jay Moore

William Morris

Robert Mossman

Fidencio Moya

Naomi Mudge

Lynn Mullenaux

Virginia Mundy

Carroll Munz

Dewey W. Murray

Michael Mutschler

Tina Myers

Dennis Nakashian

Anupam Narayan

Laurie Neidich

Joy & Carla Nelson

Toni & James Nelson

Tracy Nelson

Frank Newman

Will Newman

Mark Noethen

Albert Norcross

Jim & Sheila Norine

James Notestine

Winnie Noth

Robert Ohmart

Victor Ong

Sue Ordway

Elna Otter

Andrea Ouse

Pauline Padilla

Jeffrey Paisley

Donna Palladino

John Pamperin

Johnny Paredez

John Parsons

D. Bryce Patterson

D. R. Patterson

Roy M. Patterson

Ruth U. Patterson

George Paul

Regina Pena

William Sean Penn
Randel Penrod
Arnold Petermann
Shawn Peters
Vivian Peters
Clara Peterson
Coni Peterson
James Pilar
Theresa Pinto
Richard & Gail Potts
Jim Powers
Michael Powers
Doug Pressel
Walt Pritchard
Lowanda Pugh
David Pulsifer
John & Arlene Purbaugh
William T. Quinsler
Arlene Raban
Timothy & Sharo Raban
Arthur Reade
Holly Reck
Charles L. Redman
Neal Reidhead
Dwight & Lore Reynolds
Rose Reynolds
Linda & Raymond Rice
Daniel W. Richard
A. Richards
David "Dink" Robart
Tim Robart
Lyle Robinson
B. Elaine Rogers
Homer Rogers
Kent Rogers
Leo & Marie Rogers
Merlyn Rogers
Richard & Virginia Rogers
Wes, Pat, & Jacob Rogers
Jeff Rogers
Frank Ronco
Jeff Ronstadt
Margaret Ross

Linda Rosson
Kirk Rowdabaugh
Richard Rudolph
Mary Rose & F. Rush
Gary Russell
Sam Russell
Christine L. Saffell
David Salafsky
Richard L. Sandheger
Jim Sankey
Maria Sans
F. V. Saporito
Jen Schaffer
Mindy Schlimgen-Wilson
R. R. Schmoller
Andrew Schneller
Don Schuster
D. Sciensh
Gene C. Seeley
Mike Seidman
Angeline Serfoss
Amanda Shauger
Todd Shepard
William Sheppard
Larry & Mary Sherwood
Marjorie Sherwood
Larry & Rhond Shockley
Duane L. Shroufe
David & Rober Shuck
Ron Sieg
Victoria Sikora
M. Silva
Robin Silver
Donna Simmons
Dan & Roxanna Simpson
Florence Simpson
Jeff Simpson
W. T. & Nadine Singleton
Elaine Sisler
Rhiwena Slack
Arnold & Lore Slade
Davy Slade
Doug Slade

Ronda Slade
Wanda W. Slade
Irene Slater
Shirley Slaysman
Mary Lou Smith
Ray Smith
Susan Snetsinger
Sid Snyder
M. Solberg
Thomas Sonadres
Cynthia Soria
Lorna Soroko
Gary Spegal
Gregg & Susan Spindler
Glen & Aureli Stann
Carrie Stark
Kenny & Crystal Steed
Drew Stern
Don Steuter
Sillcie & Jeff Stevenson
Scott Stewart
Howard Stone
C. Stover
Douglas & Suz Stover
Floyd Stover
Kay Stradling
Marvin Stradling
Carol & Leon Strenkoski
Dick Stuart
Kieran Suckling
Judy Sugg
Thomas Swift
Peter Swolak
Margie Tapia
Philip F. Teisl
Rheal Tetreault
Cliff & Rachel Thomas
Paul Thomas
Craig Thompson
Milton Thompson
Paul Torrence
Ralph Trammell
Carrie Tucker

Jennifer Turner
Ann Udall
Robert G. Udall
Herb Van Slyke
Kenneth & Tam Vance
Deborah Vath
Mike Wagner
Dave Walker
Jesse & Sarah Walker
Libby Walker
Evelyn Wallace
Thomas Warfield
James M. Webb
Valora Webster
David Weigel
John Weiss
W. Welch
Manfred Wenner
Bill White
Richard White
Ken Whiting
Karen Williams
John Willis
Don Wilson
Gary Wiltbank
Jeff Wiltbank
Judy Wiltbank
Michael Wiltbank
Ricahrd Winstead
Liz Wise
Ed Wissinger
Robert Witzeman
Eleanor Wootten
Thomas Wootten
Nancy Wright
Kevin Wynn
Don & Linda Zepp
Cory Zimbleman
Carol Zimmerman

Arkansas

Sherry Balkenhol
Joel Bard

D. Blackburn
David Blackburn
Al Brooks
Alvin & Jane Brooks
Henry Dowse
Charles Gresham
Basil Kyriakikas
Jim Northum
Frank A. Roth
John T. Shannon
John Shannon
Fred Stephen
Connie Swanick
Lynne C. Thompson
T. Walker
Tamara Walkingstick
Jerry Williams

California

Seth Ackerman
Lani Adams
Evan Albright
Gary Allen
Dana Amarisa
Paul Andrade
Kelvin Askew
Mark Edward Attew
Dominique Avery
David Bakke
Jean Baldrige
Mark Balitzer
Karen Bane-Gaston
Steven W. Banning
Janet Barber
Kevin Barry
Alan Bart
Justin Bastow
Pete Batchelder
Diane Beck
Randy Benthan
Sandy Berry
Lauren Blaschke
R. J. Blinkwolt

Audrey Blumeneau
Randy Bostick
Joseph & Susan Bower
Audrey Bowers
Jo Boyard
Charles Boyce
Theresa Brady
Steve Branch
Stephen & Irene Brewer
Michael Brewster
James E. Brookshier
Robert Brothers
Michael Brown
Steve Brown
Gary Brown
Terry Bunch
Carrie Caldwell
Mary Carpelan
David Carter
Marian Carter
Steve Cassidy
Beverly Cherner
Alexander Clayton
Walter Cook
James Cooney
J. Simon Cornette
Rachel Couch
Mallory Crenin
Lyle Dahms
John Dale
D. N. Danielsen
Sandell Davidson
Galen Davis
Pat Davison
Owen Dell
Bob Denike
Lou Anna Denison
E. M. Dennis
Jerry Dewey
Sarah Diehl
Bonnie Dombrowski
Cynthia Douglas
Robert V. Dowell

Lenore Dowling	Floyd Hamilton	Harlo Lenning
Magen Dryshale	Danny J. Hamon	Emily Loen
Kathryn A. Dudley	Evelyn Harrigan	Mark Loughridge
Shawn Duke	Norma J. F. Harrison	Heather Louwsma
Marty Dumpis	Kari Hartmann	Sara Louwsma
David Duncan	Sara Hayes	Steven Luo
Colette Dupont	Todd Heinsma	Joe Machado
Gloria Durant	Dennis Heinzig	M. Magleby
Constantina Economou	M. H. Hemp	Heather Mansfield
Bruce Emerson	Tanya Henrich	Ara Marderosian
Sarah Emmerson	Nancy Higbee	Tamar Margolit
Laura Engeman	Robert C. High	Amber Martin
Brian Espy	Frances Hillyard	Charles Martin
Adela A. Fast	James Hines	Carolyn Martus
Laura Ferejohn	John Hofmann	Kanta Masters
Mildred Filiberti	Ted Hogan	Joyce McCann
Wendy Fleming	Alice Hone	Michael McFarland
C. B. Follett	Mello Dee Hrdlicka	William McKillop
Ivan Forbes	Jerry Hughes	Suzanne McMillan
Sara Foster	Michael Hughes	Camille McNeely
Charles Fox	Thomas Hunt	Don Mecchi
Dennis Freeman	Nancy Ingalsbee	Trish Meyer
Anthony Friend	Lottie Jenvey	Joe E. Miller
Bill Friend	Samuel B. Johnston	Christine & Greg Miller
Pat Frost	Stephen M. Jones	Jack Miller
Aileen Furuyama	Julia Jones-Ufkes	Pat Minyard
Ron Gaul	Natalie Josef	Ahned Mohsen
Eric Gerstung	Edna Juck	Maya Moiseyev
Robin K. Gibson	Isabelle Kay	Peter Morrisette
Alan Goggins	Mha Atma S. Khalsa	Charlie Moss
Rena Gonzalez	Mary Ann Kiger	Dave C. Mough
Richard Gordon	Diana Joni Kindwall	Bob & Jan Mountjoy
Leslie Gordon	Elizabeth Kinney	Roger Moussa
Raymond Grace	Saran Kirschbaum	Reuel Myers
Steve Graves	Kyra Kitts	Denver Nelson
Stuart Gray	Thyra Knutson	Kenneth R. Nelson
Leda Beth Gray	Jeff Koch	Beryl Ness
Anika Green	Mary Koopman	Andrea Newman
Nate Greenberg	Vanessa Kranda	David Owen
Dana Gurdling	Charles L. Krusp	Felice Pace
RaLana Gurney	Peter H. Kurtz	Elizabeth Painter
Bob Gustafson	Laura Lee	Debby Parker
Dave Hall	Edward Lemos	David Paschal

Scott Pearce
Jay & Annette Pennock
Jim Pentrack
Cheri Pillsbury
Carolyn O. Pizzo
Philip W. Poor
David Popp
Ron & Daun Powers
Ruby Price
Lynn Ragghianti
Jim Rains
Hamid Rastergar
Yvette Redler
Kristin Reed
Saelon Renkes
Don & Karin Riley-Thron
Jesse Rios
Donald Rivenes
K. Roark
Lois Robin
Mike Rogala
Pandora Rose
Stephen Rothstein
Leslie Rowe
Earl Ruffa
Kim Rusher
George Russel
Eli Sarnat
Alex Saunders
Eileen Sauppe
Rob Schaeffer
Jane Schneider
Dave Schultz
Jason Scorse
Susan Shapira
Alison Sheehey
Anne Sheldon
Brenda Sherman
Tamia Sheyner
Alison Shilling
Matthew Silver
Philip Simon
David Slater

Gregory M. Smith
Kathy Smith
Bill Snyder
Jesse Rios Snyder
Glenn Stewart
Bonnie Stoehn
Luben Stoilov
Bonnie Story
Kent Stronsmoe
Maggie Sullivan
Anna Suranyi
Mika Suzuki
Stan Swenson
Carolyn C. Taylor
Diana M. Taylor
Martin Taylor
Thomas Tereszkiewicz
Craig Thomas
Dale Thornburgh
Cheryl Tillotson
Sara Timby
Alexandra Toledo
Istvan Toth
M. Toutonghi
Kiran Turan
Cathneen Tuttle
Andrea Tuttle
Craig Usher
Judy Vance
Angelo Vassos
Phoenix Vie
Natasha Vilagi
Rosann Volmert
Ron Voss
Johanna H. Wald
Kristine Walker
David Wall
Michelle Waters
Robert D. Webb
Roland Weidenkeller
Carl & Stanley Weidert
Breana Wheeler
Wilma Wheeler

Claudia Whitnah
Scott Williams
Anthony Wilson
Lynda Winslow
James Woods
Tiffany Woznicki
Madeline Yamate
Don & Lila Young
Michael Young
Glenn A. Zane

Colorado

Charles Adams
Kelsey Alexander
Richard Alfred
Scott Balcomb
Robert Belford
Sarah Bender
Harry Benton
Carl & Nora Bernklau
William Braun
Eleanor Brickham
Tom Brinkmeyer
Merlynn Brown
Marth Brummett
Don Byers
Bob Cain
Stan Capps
Len H. Carpenter
Joy M. Caudill
George & Cathy Chandler
Alex Chappell
Kathleen Christensen
Elizabeth Considine
Frank G. Cooley
Bob Currie
Rick Cutler
David Danciger
William & Jan Daufman
Earl H. Dean
Donald Lee Delise
Mr. Stanley Dempsey
Rich C. Dever

Darlene Doyle-Stockey
Lynne Drogosz
Patrick Duffield
Marin & Leona R. Dumont
Keith F. & Eula Dunbar
Jim & Irene Dysart
Howard W. Earnest
Charles Van Epps
Harlen Feder
Kenneth Fish
William Fisher
Nancy Fishering
Michael Gates
Bill Gibson
Leslie Glustrom
William & Shirley Goosman
Mr. Larry Green
Robert P. Greybar
Sanford V. Griffin
Gus R. & Christy Halandras
Norman A. Hassig
Hugh & Jennifer Hatcher
William Heicjer
Meeker Herald
Gary & Karen Hill
Stephen Hill
Jim Himmes
Glen Horn
James Hubbard
John & Phyllis Hyrup
Thrisha Jones
Tom Kaldenbach
Kenneth Kelley
Bob Kirkegaard
John Kirkham
James & Pearl Knight
Mary & Brian Koehn
Wayne Lacovetto
Ed Lawn
Norm Lewis
Richard & Phy Lockhart
Suzanne MacDonald
Angie Many

Carl Marcus
Ron Margolis
Amy Marsh
Thomas J. McCloskey
George L. Mohar
Leno & Shirley Montover
Howard & Jeanet Moser
Robert Myers
Lori Nielsen
Dorothy O'Connell
Jim Olp
Marcia Patton-Mallory
Terry Paulson
James Peacock
Hal & Tom Pearce
Lisa Peraino
Robert & Ruth Perry
Tracy Pheneger
Patrick M. Pherren
Tom Phillips
Victor Pierson
Steve Pittel
Gloria K. Pollard
Robert Porter
William J. Post
John Randolph
Randall Rasmuffen
Robert Ray
Bob Reiling
Bob Richard
R. Richard
Robert D. Richard
Bob Richard
Tom Riesing
James L. Rose
Ted & Charles Ryden
Eloy A. Sandoval
Frank Sapio
Bill Schapley
Steven Schiff
Duane Scott
Leslie Scott
Thomas R. Sharpe

Verne & Lina Soucie
Jim Spenst
Sam Stapleton
Mr. Joseph Staufer
Don Tosha
Gene Tourville
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Roy Hazzard

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Samuel Mace

Andrew Manus

Isaac Markowitz

William Marsh

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Rex, Susan M. Winegar
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Howard Fox
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S. Smith
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Louise Cummings
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Anthony Grossman
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Linda Alverson
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Walt Armstrong
Dennis Arsenault
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Lee Harry
Bruce Higgins
Jack Holmes
Sharon Klinkhammer
Steve Kohler
Sue MacMeekan
Bill Michels
George Nickas
Jane Olson
Wes Paulson
Christopher Reichert
Jerry Sass
Steve Slaughter
Kathy Tribby
Lynn Vrlanic
Val Walker
John Weinert
M. Whalen
Michael Wood

Nebraska

Gary Hergenrader
Susan Schimmer
Ruth Wusk

Nevada

R. C. Bechtel
Valerie Buchanan
Frank R. Cervantes
Gail Durham
Gail Ferrell
Trevor & Tracy Ganske
Shelly Germann
Steven Glimp
Doug Hunt
Anna Keyzers
Robert King
P. C. Martinelli
Marian Mckenzie
Leanne Miller
Steve Robinson
Patrick Rucker
Marilyn Tomkins
Robert Vaught
Roy & Ruby Venghams
Sean & Erin Wallace

New Hampshire

Charles Baylies
George Bell
Putnam Blodgett
Jennifer Bofinger
Charles Bond
Philip Bryce
Michael Burke
Norman Charest
Richard Chase
Raymond Conley
Gibb Dodge
Tom Durkis
Kevin Evans
Rick Evena
Peter Farrell
Donna Gamache
Buhrman Garland
Walter Graff
Frank Hammond

Bruce Jacobs
Jonathan Janis
Kenneth Jordan
Keith Kidder
Charles Koch
Fred Kocher
Ted Lacey
Kyle Lombard
Quentin Mack
John Martinson
Bruce McAllister
Brooks McCandlish
Joseph McKeever
Dennis McKenney
Charles Moreno
David Noyes
David Olson
Daniel Reed
Pete Renzelman
Peter Rhoades
Jay Seavey
Bruce Sloat
Norma Sorgum
David Thompson
Robert Todd
Wayne E. Vetter
John Twichell
John Violette
Steve Walasewicz
Wayne Young

New Jersey

Diane Allen
James Barresi
Paul Barrett
Joseph Bateman
Diane Beatty
Judson Bennett
Paul Berezny
Deborah Boerner
Melani Bolyai
Ian Borden
Paul Borokhov

William Brash
Tom Brodde
Thomas Bullock
Gene Burks
Hugh Carola
Dennis Chandler
Nancy Coleman
Paul Cowie
Joseph Dunn
David Edelman
G. Ettenger
Galen E. Ettinger
Lorens Fasano
Robert Fimber
R. Fine
David Finley
Richard Goodenough
Ted Gordon
Heather Gracie
William Grundmann
Christina Harrigan
Cora Hartshorn
Kris Hasbrouck
Curtis Helm
Neil Hendrickson
Frank Hennion
Madelyn Hoffman
Lewis Howell
Christina Hurd
Mark Hynson
Matthew Immergut
Craig Kane
Constance Katzenbach
George Paul Koeck
Mary Lamielle
John Linson
Lucine L. Lorrimer
Gary Lovalo
Linda Mack
Wendy Malmid
Timothy Matthews
Steve Maurer
John Mayyyott

Bob McDowel
William Metterhouse
Nenneth Meyers
David Moore
Robin Murphy
Steven Panter
Linda Price
Stuart Rich
Paul Rodriguez
Nicole Roskos
Barbara Sachau
Dale Schweitzer
Jack Shuart
Robert Sidor
Timothy Slavin
Sunil Somalwar
Hank Suydam
Kenneth Taaffe
Linda Tatem
Douglas Tavella
Dena Temple
Luke Templin
Bob Williams
J. Worrell
George Zimmerman
Joe Zoltowski

New Mexico

Doug Abbott
Ann Alexander
Craig Allen
Debra Allen-Reid
Josefina Alvarez
Jim Bailey
James Bailey
Randy Balice
Mary Ann Baruch
Hugh E. Bearup
Joanie Berde
Jacque E. Blackman
Butch Blazer
Doug & Penny Bogart
Jess Bowman

Billy O. Branman
Jerry Brock
Kelly L. Bryan
Ysabel Campbell Luecke
Henry Carey
Elizabeth G. Chapman
Betty Jane Curry
Leslie Davis
Nelson Denman
Rich Detry
Ellen Dietrich
George Duda
Robert & Lill Dunn
Ron Ensminger
Karen Epperson
Judith Espinosa
John Fowler
Ric Frost
Delbert L. Fulfer
Charles Fuller
Sid Goodloe
Frank E. Gorskey
Randall Gray
Carrie Green
William Gross
Wayne Gyulai
Darrol L. Harrison
Bruce Hayward
Martin T. Heinrich
David Henderson
Sam Hitt
Mark Hoak
Larry Hughes
Timothy Humphrey
Abe Jacobson
Thomas Jervis
Sarah Johnson
Jennifer Johnson
Jack T. Jordan
David Keller
Ed Kelly
Suedeen Kelly
Richard Khanlain

Lane Krahl
Anthony Ladino
Gabe Lopez
Willie Lucero
Leonard Lucero
Richard V. Ludwig
Paul Luehrmann
Greg Magee
Anne Malone
Jerry Maracchini
Freddie Martin
Aron Martinez
Michael Massey
Pat Mathis
Fred C. & Wil May
Ann McCampbell
Mr. Jerry McCrea
Tracy McFarland
Julie McIntyre
Stephen Mergault
Mark Miller
Verna Miller
Tim Mitchusson
William H. Moore
Patrick Morandi
Alex Mueller
Nance Neskaukas
Rick Norris
James Norwick
Douglas B. Osborn
Manuel Pacheco
Doug Parker
Jeff Pierce
Gregory Pollak
Joanna Prukop
Don Rauch
Harold Reynolds
Eric Roybal
Deleen Ruebush
Richard Ryan
Calvin J. & B. Salars
Ben Sanchez
Pat D. Sanchez

Stephani Sandoval
Mike Sauber
Joe Savage
Melissa Savage
Hazel M. Shuck
Jack Stauder
Mary Steele
Carol Sutherland
Fred Swetnam
John Talberth
Dierde Tarr
Chancel Teague
Alex Thal
David Ther
Jerry Sue Thompson
Bruce Thompson
Lauro Vanegas
Arlene Walsh
Rhonda Ward
Jan Ward
Dave Wilgues
Marjorie Williams
Rex Wilson
Wade Worrell
Raleigh Zellers

New York

Scott Aldridge
Clifford Asdal
Richard Audette
Jordan Bain
Todd Baldwin
Wilford Bartlett
Richard Bell
Nadya Carolyn Bennett
Glen Berger
Bobbie Blowers
Herb Boyce
Bernard Braun
John Burton
Steve Callahan
Jerry A. Carlson
Marcia Carlson

Steve Collins
William Connally
James Danoff-Burg
Robert Davies
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Brian Dermody
Jane Difley
Dana Drake
John Earl
James Farrar
Richard Fox
I. M. Frellsen
Ronald Frisbee
Bill Galdstone
Edward Gammon
Cindy Garfield
David Gee
John Gibbs
Jody Gray
Ann E. Hajek
Glen Hampton
David Hawke
John Herrington
Ken Hotopp
Robert Howard
Sabrina Islam
Calvin John
Jack Karnig
Susan Keister
John Koshorek
David Kotzle
Ted Kozlowski
Peter Levatich
Lowell Lingo
Wendy Lochner
Lisa Maybee
Warren McKeon
John Miller
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Robert Moore
Bob Mungari
Aprille Nace
Gary Nelson

Evan Nichols
Rodney Nielsen
Robert Peterson
Richard Pettus
Barlow Rhodes
Jim Roberts
Monique Roberts
William Roe
Ed Schaffer
James Schuler
David Seymour
Phillip Siarkowski
David Sinclair
Vernon St. Louis
John Stratton
Robert Synowiez
Patricia Testa
Christopher Thompson
Louis Tirtito
Wayne Tripp
Sarah Waite
Fred Wilhelm
Rodman Williams
Alex Wipf
Allyn Wright
Michael Wright
Jennifer Zeh
David Zlomek

North Carolina

Stanford Adams
Weedie Barnard
Phil Bell
Erica Blackwell
Nancy Brown
Richard Brown
Leo Bunce
Kevin Carpenter
Gene Cross
Tracy Davids
Brian Fireman
Joann Fireman
Paul Gallimore

S. Andrew Gerry
John Ghent
Lessie Mae Graves
Robert Giles
Harris Gruber
Fred P. Hain
Rick Hamilton
Edward Harrison
Brian Heath
Phillip Heatherly
James B. Jones
John Kent
Donna Leonard
M. Leonard
Olivia Lim
D. Martin
Mike Massey
A. Mustian
James Padgett
Brett Pendergrass
Ethel Pittman
Derek L. Puckett
Robert Reiman
Donald F. Rogers
Stephen P. Schmidt
Aron Sebastian
Terry Seyden
Jill Sidebottom
Walton Smith
Deborah Steward
Robert Thatcher
Ron Thigpen
Michael Thompson
Robert Trickel
Diana Underhill
Ralph Willard
James Yount

North Dakota

John Brauner
Dean Hildebrand
Dave Hirsch

Larry Kotchman
Joe Maxwell

Ohio

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Daniel Balser
Joel Berry
Pamela S. Blackburn
Robert Boley
Michael J. Budzik
B. Burke
Brian Burke
Richard Cappell
Greg Crandall
John Dorka
Robert Endebrock
Judy Fink
David Fleischer
Jeff Frontz
Tammy Frye
Margaret Garwook
D. Geglein
Stephanie Glazer
Robert Hampel
Margaret Harwreak
Betty Jean Herner
Tim Humprey
A. Lacy Johnson
Lacy Johnson
Robert Lamoreaux
William Lebold
Michael Littlejohn
Frank Luppino
Steve McKee
Thomas Morban
K. Niese
Galen Oakes
Peter Oros
Gilbert Papsy
B. Ramsey
Richard Ramsey
Deb Reed
C. Richards

Chris Richards
Delores Rogers
Amanda Schackow
James Scheetz
Siobhan Wolf Shaffer
J. Slavicek
James M. Slavicek
Mary Smallsfeed
Kathleen Smith
Jim Stafford
Thomas Stanley
A. Stone
James Suhanic
Stanley Swierz
Robert Tapeman
Algimantas P. Valaitis
Becky Violey
Joseph Vitti

Oklahoma

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Sancho M. Dickinson
Jacob Frank
Jack Gobin
Pat Mcdowell
Bill Ross

Oregon

Hilary Abraham
Sherry L. Adams
Dennis Ades
Thomas P. Ady
John Aguirre
Steven Akehurst
Nate Alexander
Tena Alvarez
Ed Alverson
Bob Amundson
Arvid Andersen
Debbie Anderson
Don E. & Pat Anderson
Donald J. Anderson

Jim Anderson
Zach Anderson
Martin Andre
Bret & Doris Armacost
Jerome & Jane Arnold
Larry Aschenbrenner
Kathy Askren
Mary M. Atkinson
Alan Ayres
Dale Badrick
Barry B. Bai
Ric Bailey
James Bailey
Tim Bailey
Ric Bailey
James Baker
Jerry Baker
Bruce Ball
Cindy Banzer
Jamie Barbour
Stanley Barg
Lyle Barkman
John R. Barnes
Rick Barnes
Dan Barnett
Richard Barnette
Donald & June Barnum
John Barry
Edward & Bob Bartell
Conrad Bateman
John & Patricia Bates
Robert A. Batty
Byron Beach
Joy Belsky
LeRoy Bennett
Leslie Benscoter
Ken Benson
Ted Berg
Lars Bergstrom
A. Berier
Thomas Berkemeier
Daniel Berman
Donald Bettis

Ann Bettman
Gary Betts
John & Linda Biehl
Ken Bierly
Mack Birkmaier
Tom Birkmaier
Cal & Lorna Birrer
Dan Bishop
David Bishop
Neil Bjorklund
Erin Black
Scott Black
Christopher & J. Blake
Larry Bliesner
Richard Bloom
Dick Blum
Mike Blumm
Mark & Vicki Bolton
Donald & Donna Bond
Dale Bonnell
Tracy Bosen
Cassandra Botts
Gerald Bowerly
Grant Bowerman
Sue Bowers
Sandy Boyce
Jack Boyd
Jim Boyle
David Boyles
Durward L. Boyles
Albert Bradford
James & Doris Brady
Mike & Kathy Brandis
Bruce Brandt
William D. Brand
La Dora Brasel
David M. Braun
John & Lynne Breese
Lynne Breese
Eugene Brick
Dave Bridgwater
Chris Broadfoot
D. Brodie

Bob Brown
Gary Brown
Jason Brown
Mark Brown
Marvin Brown
Rick Brown
Ronald Brown
Brett Brownscombe
Charlie Bruce
Virginia Buck
Amy Buffum
Charles H. Burley
Chuck Burley
Tom & Inger Burns
Steven C. Buttrick
Bruce Byerly
David Byrnes
Jean Cameron
Vera Campbell
Robert Carl
Don Carlton
Vanelle Carrithers
Robert G. Carson
John Carter
Steve Carter
Richard D. Cartwright
John A. Cason
Bill Castillo
Juine Chada
David Chamberlain
Rodney & Kimber Chambers
Sheila Chambers
 Richard L. Chapman
Frances Chapple
Frank N. Chase
Harold Chase
Arthur & Annett Cheatham
William J. Cherry
Rod Childers
David Childs
Phil Chlopek
Christopher Christie
Victoria Churchill

David Clairborne
Keith Clark
Laura Cleland
Sidney N. Clouston, Jr.
James Coburn
Noelle Colby-Rotell
Larry D. Cole
Paul, Vicki Conable
James Conlay
Robert A. Cook
Todd & Barbara Cooley
Eric M. Coombs
Katheryn Cooper
Steve Corfield
Grant L. Cornelius
Bette Coste
Doug Cottam
Karen Coulter
Caroline Cox
Michael Cramblit
Tim & Cynthia Cramblit
John Cramer
Gary Cremer
Dave Crider
Ernest Cristler
Steve Cross
Joseph Crowell
Nancy Crumpacker
Gordon Culbertson
Charles & Mary Culver
Ron Cunningham
Tim Cuthbertson
Keith Cyrus
Paul Czemerys
Chris Daggett
Bob Dale
Kim Davenport
Duane Davey
Jeff Davies
Shannon W. Davis
Bert Davis
Tim Davis
Robert P. Davison

Michael Dean
Major Defoe
Garth & Cathy De'garlais
Susan Delles
Marci Denison
Ray Denny
Mark Desmarets
Lisa Devaney
Paul D. Dewey
Alan Dickman
Penney Diebel
Jeff Dillon
Tom Dimitre
Robin Dobson
Wanda Dobson
Paul Doescher
Mark Dohrmann
Jacquin Dole
Eric Dolson
Deanna Donaca
Jim Dovenberg
Linda Driskill
Robert Drummond
Barbara Dudman
Marianne Dugan
Julia Dugan
Bruce Dunn
Jack & Imogene Dunn
Jim Durbin
Robert Dusenbery
Laurence Dyer
Gregory J. Dyson
James Eblin
Kelly R. Edwards
Ron Eggleston
Paul F. Ehinger
Richard Eiguren
Paul Eisenberg
Don Eixenberger
Lyle Ellickson
Cal Elshoff
Clint Emerson
Nadine Emery

Paul Engelmeyer

James C. Engelstad

C. Enyart

Kimbell Erdman

Arthur Erickson

Robert Ervin

Wayne Estabrook

Gary Estes

Dan Evans

Louisa Evers

E. Lucille Farr

Susan Fay

Helen Felbick

Don Feldmann

Brent Fenty

Carol Ferguson

Dan Ferguson

Denzel Ferguson

Charles J. Ferranti

Senator Ted Ferrioli

Shelby Filley

Stephen D. Finlayson

Melissa Finn

Edwin & Laurene Fitzjarrell

Donald Fontenot

Bruce Forbes

Sandy Force

Dick Ford

Scott Forrester

Walt Forsea

Daniel Forsea

Toni Foster

Rachel Foster

Brad Fowler

Gary Fowles

Ken French

Don & Emilie Frisbee

Cheryl Fuller

Georgia Gallagher

Richard L. Gambrall

David & Judith Gardine

John H. Garren

Thomas & Lana Garrett

Kalvin B. Garton

Sharon Gatlin

Frank Gearhart

Larry Geiber

James C. Geisinger

Jim Geisinger

M. J. Gemmet

Walt Gentis

Rick George

Randy Ghormley

Richard Gibson

Jerzy Giedwoyn

Kent Gill

Don & Joann Gilliam

Robert J. Girdner

Susan Glarum

Cheyenne Glasgow

Alicia Glassford

Jared Goddard

Jerome & Waltro Goertzen

Don & Ellen Goheen

Peter Goldman

Daniel L. Goldy

Dan Goltz

Pete Gonzalves

Tom Goodall

Dan & Marilyn Graham

Richard Granger

Bill Granning

Clark Gray

Barbara Green

Jim Greer

Norma Grier

Mary Griffin

J. Groom

Mike Gross

Dean Guess

Tom & Maggie Gunn

Carol & Herma Gunnels

Kenneth & Mary Gustafson

Jim Gustafson

Lester R. Haglund

Carl L. Hagstrom

Eldon Haines

Howard A. Hall

Lexie Hallahan

Karl C. Hallstrom

Jessica Hamilton

Larry Hamilton

Ray Hamilton

Paul Hammond

Michael F. Hanley

Nancy Hanna

Dorothy Hannigan

Susan Hanscom

B. Hanson

Mary Hanson

Richard Hanson

William Hanzen

Robert & Shalen Hargreaves

Norman R. Harris

Robert Harrison

James W. Hart

Walter T. Haswell III

Judy Hatton

Eugene R. Hawes

Clayton Hawkes

Gary & Collee Haynes

Michael Hayward

James Hedgecock

Doug Heiken

Anita K. Helser

Kathleen A. Helser

Richard & Anita Helser

Elwayne Henderson

Lebron Hendon

Sarah Hendrickson

Mark Henjum

P. Sydney Herbert

John Herbst

David & Sandra Herman

Helen Herman

David Herr

Pam Hewitt

L. R. Hiatt, Jr.

Claire Hibler

Jerolee N. Hickey

Dennis V. Higgins

Sue Hinton

Mark E. Hitchcox

Kelly Hockema

Gary & Maggie Hoeppner

Mary Holbert

E. E. Holder

Carmelita Holland

Todd Hollis

Arthur Holmes

Steve Holmes

Irwin Holzman

Chad I. Honl

John O. Hooson

Scott W. Horngren

Zane Horowitz

Kay Houck

Harold Houghtelling

Reis Hoyt

Laura Hudson

Wendy Hudson

Warren Hudspeth

Patrick & Donna Hughey

Amie Huish

Jewel Hult

George Hutchinson

John & Tammy Hyland

George Ice

Jerry Igo

Emery John Ingham

Frank Isaacs

Gary Ivey

Joan Jacobsen

Carol Jacquet

Irene James

Lisanne Pearcy

Ginny Jayne

Aaron Jennings

Paul Jepson

Becky Johnson

Diane E. Johnson

Elizabeth K. Johnson

Jesse F. Johnson

John Johnson

John & Brenda Johnson

Kathleen J. Johnson

Jeffrey Johnston

Parker Johnstone

Russ Jolley

Herbert & Virgi Jones

Denise Jones

Ed Jones

Ted Jones

Callie Jordan

Dorothy Josellis

Susan Joshua

Steve Kadas

Garth Kahl

Richard & Trudy Kalac

Peter & Lorrain Karassik

Oscar & Sharon Kay

Gery Kazda

Donald & Trudy Kearney

Floyd Kednay

Lloyd T. Keeland

Roy Keene

Robert L. Keeney

Mike & Joanne Keerins

Kent Kelly

Bill Kelso

W. Dean Kendall

Lloyd Kendrick

Tim Kerns

Andy Kerr

Merle Keys

Kevin Kilduff

Ed Kimball

Robert P. Kingzett

Ann Kinnaman

Maureen Kirk

Anita Kirkaldy

Mellissa Kirkland

Gary Kish

Dennis & Joan Kizziar

Walt & Patty Knapp

James & Judith Knapp

Devon Knutson

John Koenig

J. Pierre Kolish

John Kowalczyk

Paula Kreger

Bob Krein

Ralph Krellwitz

Mary Krenowicz

David Kucera

Leigh Kuhn

Paul Kunkel, Jr

Ronald Kunzman

E. A. Kupillas

Philip Lanfear

Alfred & Doris Lang

Jeff Lang

Doug & Pat Larsen

Larry Larson

Patricia A. Larson

Bruce & Frances Lattin

Rhett Lawrence

Sam Layman

Barbara Lee

Duane & Marian Lee

Georgia M. Lee

Patrick Lee

Jack Leishman

Spencer Lennard

Steve Lewis

Jay Lininger

Connie Linsdale

Mona Linstromberg

Clyde Alan Locklear

Bev Loennig

Carol Logan

Patricia Loveland

Thomas Lovlien

John Lowe

Marilyn Lowe

Robert A. Luna

Ted Lyster

Nancy Machugh

Duncan S. Mackenzie	Bob Meinke	Jay Nelson
Joseph & Connie Madar	Charles Meslow	M. Nelson
Ron Maertz	Robert C. Messinger	Mitch Nelson
Clifford Mann	Brian Richard Metke	Mitchell G. Nelson
Mari Margil	Mark & Marie Metzdorff	Mark Newbill
William & Carol Mark	Christopher C. Meyers	Bruce Newhouse
John Maroney	Holly Michael	Carl Newport
Norm Marsh	Charles Middleton	Frazier Nichol
Dave Marshall	Virgil Miller	Marvin Nichols
Stan Martindale	Robert Miller	Craig Nielsen
Marvin Maxwell	Mike Miller	Fred Nilsen
Thomas May	Jeff Miller	Ranei Nomura
Edward & Marily Mayers	Terry Miller	James D. Noteboom
Larry Mayes	Glenn Miller	Karl E. Nulton
Rynn Mazur	Randy Mills	Carol Nygaard
Michael McAllister	Roy Milner	Mark Nystrom
Ian McAndie	Elizabeth Mitchell	Richard Oberdorfer
Mike McCann	M. J. Mitchell	Robert Oborne
Scott McCaran	Larry R. Mittnacht	Mary O'Brien
Dave McClain	Ray L. Moles	Mary H. O'Brien
Gerry McClain	David Monk	Mike Obymako
Greg McClarren	James Monteith	Mike & Nancy Obymako
Albert McCollam	Marty Moody	Steve Odell
Evelyn McConnaughey	Chris Moore	Paul Oester
Bruce McCullough	Marilyn J. Moore	Sara Olsher
Glenn McDonald	Robert L. Moore	Charlie O'Neal
Peter McEvoy	Tam Moore	Elizabeth O'Neill
Tim McFetridge	Wayne Moro	Charlie & Jan O'Rorke
Michael McIlrath	Bob & Terry Morse	Douglas & Roxan Osborne
Mike McInnis	Guy Mount III	George & Rhonda Ostertag
John & Lelia McIntire	Alan Mudge	Robert Otteni
Albert D. McKenzie	Dan Mulligan	Stephan Otto
Katheryne McKenzie	Mrs. Steve Mullin	Dave Overhulser
Roger L. McKinley	Bob Mullong	Jeff Oveson
Rebecca McLain	Andy Munsey	John Owen
Dimetra & J. McLain	Ronald Murphy	Stan Owen
Jim McLean	Victor P. Musselman	Dwight Owens
R. C. McNeil	Dennis Myhrum	John & Madeline Pagano
Brian McNerney	Cheryl Neal	George Page
Michelle McSwain	Edward Needles	Edward Page
Sarah Medary	Grace E. Neff	Jeff & Susan Pape
Beth Medler	Richard Nelsen	Stephanie M. Parent
Johnny Medlin	Dick Nelson	Rick Parker

Neal C. Parsons

Tom Partin

Kerry Paulson

John Peaks

Ed Pearson

Jack Peasley

Eric Perkins

Mike Perkins

Peyton & Ruth Perkins

Wayne B. Persons

Larry Petersen

Marilyn Peterson

Joe A. Petrovich

Donald H. Phillips

Therese Picado

Phil Pinney

Russ Plager

Harold & James Pliska

Don Podrabsky

Catherine Poncil

Delores Porch

David Post

David Potter

Michael Powelson

Laurie Power

Daniel Powers

Tony Pranger

George & Alicia Prigmore

Karl Puls

David A. Pyke

David & Beth Quick

Bob Quitmeier

Hans Radike

Iral Ragenovich

Ruth Raizin

Teri Raml

Sheri Rand

Alan A. Rappleyea

Mark Rasmussen

Don Rayborn

Kevin & Patrici Rea

Elizabeth Redon

Phillip Reed

W. R. & Janet Reed

Erika Reesor

Diane Reimers

Richard & Chery Reinertson

Troy Reinhart

Byron Rendar

Gary Rhinhart

Chuck Rhodes

David & Coralie Rhoten

Russell S. Ricco

Bob Rietman

Doris J. Riggs

Robert S. Riley

Robert & Ann Rissberger

Asante Riverwind

Clarence & Dolores Robart

Dvora Robinson

Thomas & Donna Robinson

Jean Rodgers

Maggie Rogers

Dan Rohlf

Noelle Colby Rotell

Dave & Janett Roth

Lilijoy Rothstein

Renee Roufs

Mary Rounds

Jim Roy

Skip Royes

Roy M. Runco

Paul Runquist

Jerry Russell

Ken Rutherford

Fred Ryan

Irene Saikevych

Bill Sanowski

David A. Sauer

Anne Saxby

William & Judy Scally

Benjamin Schafer

Jack Schaffer

Patrick Schatz

Jennifer Schemm

Owen Schmidt

Guenter & Erika Schoener

Edward Schoor

David & Francoi Schreiner

Reid Schuller

Larry Scofield

Donald W. Scott

Norm & Cheryl Scott

Steven Scott

Wayne & Marlene Scott

Mary Scurlock

Darwin Secord

Jim Sedell

Lloyd Seely

Joe Serres

M. Ray & Bonnie Sessler

George Sexton

R. D. & Karen Shadley

Patrick & Tamar Shannon

Kathy Sheehan

Craig Shinn

Dan B. Shoop

Terence Shumaker

Ralph Siegfried

Greg Sieglitz

Alex Sifford

Ronald E. Siler

Gene Silovsky

Annette Simonson

Bill Sisson

Carol D. Skerjanec

R. William Skinner

Robert M. Skinner

Trygve B. Sletteland

Gerald & Connie Sloper

Rosalyn Smarr

David Smerski

Chris Smith

Florence E. Smith

Gary Smith

Matt Smith

Michael & Glenda Smith

Pat Smith

Roger Smith

Ryan Smith

William Smith

Paul Smoland

Donald Smpson

Sally Snyder

Mark Snyder

Dee Southard

Maeve Sowles

Glen H. Spain

Ida Spaulding

H. Grant & Debra Spies

Linda J. Spillum

Beverly Springer

Keith Squire

Janice Staats

Naomi Stacy

Julie Stangell

Shannon Starratt

Gail Stater

Tom Stave

Chris Stecher

Trygve P. Steen

William Steers

Beth Steinberg

David Stengar

Dale Stennett

Linda & Carl Steppan

Dalton Stewart

Don Stewart

Loran R. Stewart

Lorna Stickel

Karen Stingle

David Stitt

Dan Stokes

Joseph L. Stone

Trevor M. Stone

Rex Storm

Daniel Stotter

Benjamin B. Stout

Sally Streeter

Don Stroeber

Leonard & Linda Sundval

Jerry J. Sutherland

John & Judy Svoboda

Marvin Swaggart

Susan Swatek

R. Taber

S. Tamiesie

John Tanaka

Ed Tarnasky

Trevor Taylor

Dennis Taylor

Wayne Teschner

Doug Thackery

Toby Thaler

Greg & Arlene Thomas

James Thompson

Paul Thompson

Everett & Eva Thornburg

John Thornton

Dennis Thorsen

Avery Gary Tittle

Trudy Toliver

Nora Tomlinson

Pepper W. Trail

Bruce Troyer

Gerald Trussell

Phil Turrell

Laurence Tuttle

Dee & David Tvedt

Ed Uebler

Barb Ullian

Joseph Vaile

Carlos & Sylvia Valdez

Maritza Valle

Ben Van Camper

Walt Van Dyke

Carol Van Strum

Phil Vanbuskirk

Robert Vancreveld

Roberta Vandehey

Dick Vander Schaaf

Opal L. Vankommer

Kathryn Venator

Gregory Vik

Lucy & Lawren Vinis

Patrick Voigt

Derek Volkart

Liz & Brian Vollmer-Buhl

Sue Vrilakas

Bill Waddel

John & Claudia Wadsworth

William Wadsworth

D. Kent & Gail Waggoner

Lisa Wale

Dick Walker

Larry Walker

Jack Walsh

Jack Walstad

Elden Ward

John Ward

Fred Way

Walter G. Weagel

D. R. Webb

Ginger Webster

Jim Webber

Laura Weeks

Bill Weide

Sharon Weinberger

Ted & Tami Weitman

Ray & Bobbie Wells

Vonda Welty

Mindy Whaley

Robert Whittier

Thomas Wiemann

Kathleen Wilber

John Williams

John D. Williams

Joyce Williams

King Williams

Tucker Williamson

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John & Hannah Wilson

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Shannon Wilson

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Mike Blumenthal
Chris Bobick
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Robert Connor
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William Cook
James Crockett
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I. Glendenning
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Barry Rose

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Howard Schmouder
N. Schneeberger
Albert Schultz
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Bob Shipman
Rob Shipman
Leonard Skultety
Stanley Soberi
Jeff Spako
G. Stamm
John Stankiewicz
David Steckel
Pauline Steinmeyer
Delton Stiles
Elvie Stiles
William Stiteler
Robert Swindell
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David Trost
Harry Tucci
James Uhl
Bob Wawrousek
Robert Weaver
Adriann White
Robert White
Glenn Whitmire
Jonathan Wirth
Grover Wolf
Dennis Yoder
J. Yoder
Joseph Young
William Zahuranac

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Bob Schowalter
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A. Mesman
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Shashank Nilakhe
Joe Pase

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Sims Sandra
Jim Settle
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Bobby Stark
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Jennifer Walker
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James Biser
Bob Brister
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Steve Deacons
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A. Joel Frandsen
Craig Hawke
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John Kimball
Robert King
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Jim Matson
Steve Munson
Wes Odell
Gary Orr
Lee Peterson
John Peterson

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Christy Rose
Robert Russell
David Schen
Jeff Schramm
Tom Tidwell
Jack Troyer
George Weldon
Dick Wilson

Vermont

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Tom Anderson
David Bailey
Todd Barker
Patrick Bartlett
Daniel Batchelder
Dale Bergdahl
Richard Bizzozero
Nelson Blackburn
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Barbara Burns
Bob Burt
Harry Chandler
Gregg Christie
Paul Council
Clarence Croft
Willis Curtis
Dottie Dubey
Chuck Eaton
Bernard Folta
Noel Fritzinger
Tom Gray
Clay Grove
Trish Hanson
Harry Hayden
John Hemenway
Ray Henderson
Robert Hill
Bob Hoffman
Galen Hutchison
Angelo Incerpi

William Jones
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William Kinsley
Jay Lackey
David Leavitt
Delwin Lewis
David Mance
Linda Matteson
John Meyer
Dolly Miller
Ryle Miller
Sam Miller
Robert Mills
Leanne Moorman
Ross Morgan
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Scott Pfister
David Potter
Thomas Ragle
Gloria Rapalee
Bruce Richardson
Charles Richardson
Scott Rowden
Sandy Savage
Robert Seniff
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Klinton Wigren

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Brendan Wittaker
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Wanda San Jule
Rosemarie Sawdon
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Everett See
Dee Dee Sellers
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Dr. Tcheslavskaia
Pat Therrien

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Tim Tigner
I. Fred Traw
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Tesia Williams
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Allen Yankey

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Susan Bacon
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Jeff Barrett
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Jim Berry
Bill Bidstrup
Joe Bigas
Carl Bjelland
Janine Blaeloch
Keith Blatner
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Ray A. Borden
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Gene Brain
Hal Branscom
Bill Brazelton
Shari Brewer
Allyson Brooks
Vonda Broom-Parris
Craig Brougher
William Brown
Richard Brown
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Stephen Bupp
Kevin Burgess
Ryan Burkett
Dennis Burmeister
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Clinton L. Campbell
Pamela Campbell
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Tim Coleman
Mike Collins
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Carolee Colter
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Al Cook
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William Corbin
Arvilla Corey
Kaleen Cottingham
Randall Courtney
Ted Cowan
Rex C. Crawford
Kathy Creahan
Fred Cunk
Chuck Cushman
Norman Danielson
Richard Dart
Jerry Davidson
Bruce Davies
Robert & Evelyn Davis
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Ruth Deery
Neal Degerstron
Tony & Marilyn Delgado
Ed Depurt
Gene Derig
Dan Dewald
Bob Dick
Jim Dipeso
Charles Downen
Scott Dungan
Jim Durkos
Stephanie Durman-Matheny
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Lanette M. Earnhart
Fred Ebel
Millard D. Eckert
Vicki Edwards
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Hans Ehlert
Ezra Eickmeyer
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Gordon Ellis
James Ellis
John Eminger

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Fran Forgette

Dale K. Fortune

Jeannine Frazier

Bob Freimark

Lawrence Fricke

Bob Friemark

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Mike Gagner

Howard Gains

Dean Gaiser

John E. Galley

Len Gardner

Gary Garrison

Walter Gary

Edward E. Gates

Nick Gayeski

Chris Gebhandt

John Geyer

John Gifford

Joe Ginsburg

Fred Girtman

Linda Givler

Tim Godfrey

Michelle Goling

James P. Golingo

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Shaune Gramlow

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Mark Grandstaff

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Jack & Jacpue Green

Matt Green

William Green

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Bob Groncznack

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Ray James

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C. J. Jelmberg

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Charles Kehl

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Lorraine Kile

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Glen Klock

George Koehler

Dale Kolbe

Ken Konigsmark

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A. R. Kruckeberg

Jordan Krug

Deanna R. Kuhn

Henry Lagergren

Peter Lagerwey

Tonya Lander

Mark Langston

Russ Larsen

Ray Lasmanis

Justin Lavadour

Mark Lawler

Bonnie Lawrence

Nathaniel Lawrence

Sam Layman

Hoa Le

John Leary

Renee Leask

M. Delphine Ledoux

Mike Lee

Rose A. Lee

Mark Lehinger

John Lehmkuhl

Ryan Leland

Tom Leonard

Mirian Lewis

Yale Lewis

Joseph Leysath

Phil Loe

Peter Loft

Robert Lopresti

Bryon L. Lorenz

Rob Lovitt

Louise Luce

Karen Lucei

Thom Lufkin

Guy Lusignan

Larry Luton

Dean Lydig

Doug Mace

Frith Maier

Mike & Chris Mallon

Vernon Marll

David Martell

Anne Martin

Carol Martinez

Earle & Shirlee Marvin

Jennifer Mast

Mark Matthies

Linda Maxwell

Elizabeth Mc Dowell

Mike Mc Glenn

Loren S. Mc Govern

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Mark McCann

Bryan McConaughy

Scott McCorkell

Linda McDaniel

Pat McElroy

Albert McFarland

Rick McGuire

Ed McHugh

Jim McLean

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W. B. McPherson

Bob Meeks

Robyn Meenach

Hal Meenach

Joe Mentor

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Mike Miraglio

Wayne Mohler

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Jeffery Moore

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Don Nelson

Gale Nelson

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Dale Nichols

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Chap H. Phillips

Mike Phillips

Phil Piazza

Timm E. Picknell

Sheldon Pifer

Clinton Piper

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Marlene Renwyck	B. Smith	Mark Vetter
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Joe Williams
Morris Williams
Jerry Williamson
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Norman Winn
Margo Wolf
Allan Wolfson
Charlie Woodruff
G. R. Woods
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Kerry Bledsoe
Myra Bonhage-Hale
Richard Bosley
Jim Bowen
Bill Bowling
Bruce Brenneman
Barbara Breshock
Michael Burns
Helen Butalla
Linda Butler
James Circle

William Coffindaffer
Dr. Charles Coffman
Charles Coffman
Phyllis Cole
Samuel Conley
George Constantz
Julia Cook
Jack Cromer
James Crum
Sam Cuppett
Robert Daoust
Edward Dauchess
Eileen Day
Norman Dean
Michael Demchik
Keith Dix
Gus Douglas
Dennis Dunham
Chad H. Dye
C. Randall Dye
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Matt Evans
James Evans
Virgil Falloon
Pete Filler
Don Flegel
Paul Flippin
Gary Foster
Nancy Friend
Allan Glasscock
Charles Glick
Barry Glick
Paul Goland
Kate Goodrich
Roger Green
Jan Hacker
John Hall
Howard Hardy
Marion Harless
Jamesq Hayes
James Hayes
Clark Haynes
Clark Haynes

Austin Hinkle
Sherri F. Hutchinson
David Ingram
Margaret Jenness
William Judy
Willliam Judy
Bill King
Beth Kniceley
Ray Knotts
Emil Knutti
James Kotcon
Edward Kraynok
John Landolt
Delmar Lough
William Macdonald
Harry Mahoney
David Marsh
Larry May
Tom Merril
Donna Mitchell
H Moore
Ward Moyers
Dwight Moyers
Hubert Moyers
Coy Mullins
Roy Nutter
Hazel Oldland
Jennifer Ours
Dan Parker
T Patrick
Tom Pauley
Harry Pawelczyk
K Plitt
Doug Ramsey
Rus Richardson
Natalie Rutledge
S. Arthur Rybeck
John Sanders
Butch Sayers
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George Schell
Bruce Schick
Roger Sheppard

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Pete Stemple
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Karen Sykes
Shalom Tazwell
Eugene Thorn
Patrick Tobin
Douglas Toothman
William Tusing
Jim Vangundy
Charles Vetter
Donald Wagner
George Walburn
Dick Waybright
Lester Whitecotton
Paul Wieber
Scottie Wiest
Johnny Wilkins
Ronald Wilson
David Workman
Arthuer Yagel
Arthur Yagel
Cathy Zivkovich

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Tim Bacon
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Don Bartels
Leo Baur
Jim Berlin
Frank Bremser
David Brost
Richard Camp
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Cathrine Cerness
Jay Cravens
J. Cruse

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Andrea Diss
James Drescher
Dennis Fincher
Mark Giese
Jeff Groeschl
Tony Hallman
Lee Hanks
Pete Hilgers
Paula Kamps
Robert Kasten
C. Kelly
Phil Knudson
Dan Kretz
Walter Kuhlmann
J. Kyhl
Bill Landwehr
J. Larson
Jane H. Larson
C. Lettau
Chris Lettau
Peter Luxenhofer
M. Marie
Jim McDonald
B. McNee
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James Meunier
Terry Moore
Rudy Nigl
Louise Plaskey
Willa Pledger
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William Seybold
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Michael Smith
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Edson Stevens
Craig Ver Kuilen
Allan Waelchli
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Daniel Weiss
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Carroll Zietlow

Wyoming

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Bill Crapser
Justin Gentle
Kniffy Hamilton
Brian Kelly
James Marra King
Les Koch
Bryce E. Lundell
Paul Miller
Roy Reichenbach
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K. Nielsen
G. Oliver
Shane Sela
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Italy

Alberto Cozzi

Chapter 7
Glossary
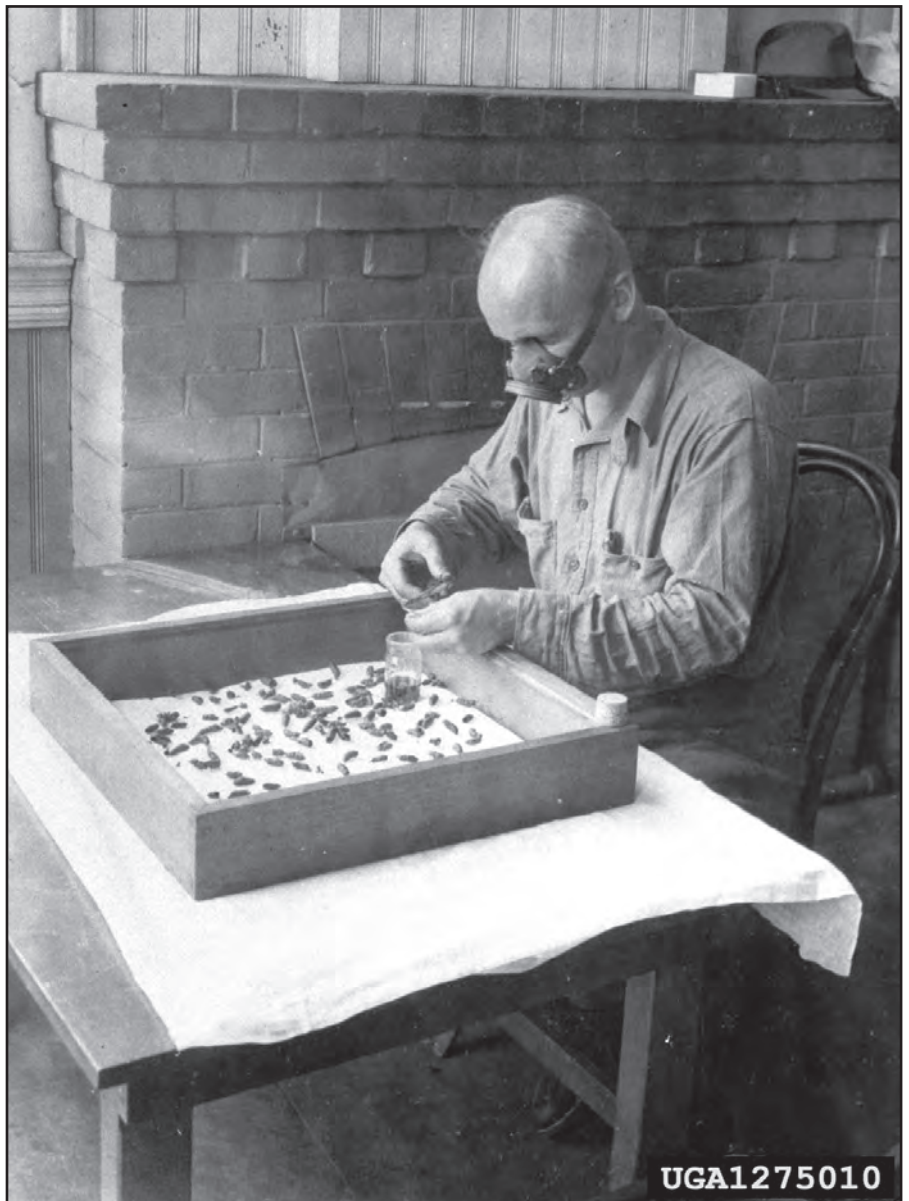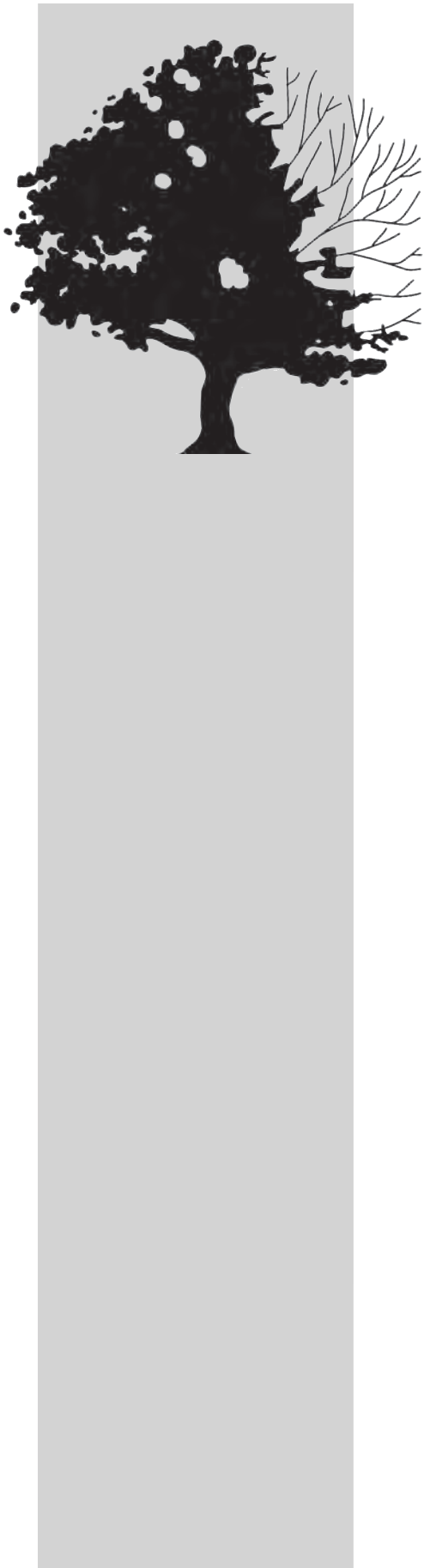
Figure 7-1. *A respirator prevented inhalation of wing scales and fine hairy particles from gypsy moth life stages.*

Chapter 7 Glossary

Figure

Terms are defined as they pertain to this Supplemental Environmental Impact Statement (SEIS).

A

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

acetylcholine — compound released at nerve endings, active in the transmission of the nerve impulse

acetylcholinesterase — enzyme that occurs in nerve endings and prevents accumulation of acetylcholine; acetylcholinesterase inhibition results in acetylcholine accumulation, which impairs the nervous system

acinar-cell adenomas — type of benign tumor

actinomycete — any bacterium in the order Actinomycetales, which contains filamentous branching bacteria of the genera Actinomyces and Streptomyces

active ingredient — (a.i.) toxic part of an insecticide formulation

acute exposure — single exposure or multiple exposures occurring within a short time frame (24 hours or less)

acute toxicity — potential of a substance to cause injury or illness in a single dose or in multiple doses over a period of 24 hours or less

adenoma — benign epithelial tumor; glandular

additive effect — combined effect of two chemicals is equal to the sum of the effect of each chemical alone. The effect most commonly observed when two chemicals are administered together is an additive effect

adjuvant(s) — formulation factors used to enhance the pharmacological or toxic effect of the active ingredient

absorption — tendency of one chemical to adhere to another material

adverse-effect level — (AEL) signs of toxicity that must be detected by invasive methods, external monitoring devices, or prolonged systematic observations. Symptoms that are not accompanied by grossly observable signs of toxicity

AEL — acronym for adverse-effect level.

aerobes — organisms that require oxygen.

aesthetic damage — undesirable change in appearance

Agricultural Research Service (ARS) — USDA agency that develops the means to protect trees in forests, parks, yards, and other nonforest environments; conducts research to support activities against the gypsy moth

a.i. — abbreviation for active ingredient

alkaline — having a high pH; a basic solution, compared with an acidic solution

allergic reaction — situation where a pre-exposure of the chemical is required to produce the toxic effect via an antibody

alopecia — hair loss

alternative — one possible way to accomplish a proposed action; a way to manage the gypsy moth in the United States

amino acids — relatively simple carbon-nitrogen molecules that are the subunits of proteins

amphiphod — any of the various small crustaceans in the order Amphipoda, with laterally compressed bodies found primarily in aquatic habits; examples are sandhoppers, beach fleas and skeleton shrimp

anaerobes — organisms that do not require oxygen

analogy to other compounds — using data on one set of compounds to predict the activity of another set of compounds

anemia — decrease in the concentration of red blood cells in whole blood

Animal and Plant Health Inspection Service — (APHIS) joint-lead agency for this environmental impact statement on the gypsy moth; the USDA Agency that enforces national quarantine, coordinates with States on the National Gypsy Moth Survey, provides assistance to States to eradicate isolated infestations of the gypsy moth on 640 acres or less, develops new methods to improve gypsy moth quarantine and eradication practices, and conducts technology transfer activities

anthelmintic — compound used to rid an organism of parasitic worms

antibodies — large protein molecules that interact with antigens and deactivate antigens

antigen — substance capable of inducing an immune response

APHIS — acronym for Animal and Plant Health Inspection Service

aplastic — pertaining to or characterized by aplasia– the lack of development of an organ or tissue, or of the cellular products from an organ or tissue

aplastic anemia — form of anemia that is difficult to treat

ARS — acronym for Agricultural Research Service

arthropods — large group of invertebrate animals that includes insects, spiders and crustaceans

artificial spread — spread of the gypsy moth by other than natural means, for example, by insect life stages attaching to and being moved on recreational vehicles, automobiles, nursery stock, outdoor household articles, and cargo

Asian strain — refers to strains of the gypsy moth originating in the Far East, which have some females that can fly, and may have the capacity to establish in a broader host range, be larger, and hatch earlier than the European strain

assay — a test (noun); to test (verb)

atrophy — decrease in the size of a cell, tissue, or organ, often associated with exposure to a toxic agent

B

Bacillus thuringiensis (*B.t.*) — bacterium; found in most of the world useful in regulation and/or control of insect populations. This microorganism produces several agents (toxins) active against insects

Bacillus thuringiensis **var.** *kurstaki* (*B.t.k.*) — scientific name of a bacterium that is specifically pathogenic to caterpillars of many moths and butterflies; the active ingredient in biological insecticides sold under the trade names Dipel, Foray, and Thuricide

basal area — cross-sectional area of a tree determined from the diameter of the trunk at breast height; the total area of ground covered by trees measured at breast height

benchmarks — results of toxicological tests, such as LCD or EC_{50} values.

beneficial organism — any organism that eats, parasitizes, or regulates in some way populations of other organisms that are pests

benign — not malignant, not recurrent, favorable for recovery

benthic — pertaining to the sea bed, river bed, or lake floor

beta-exotoxin — proteinaceous toxin in some forms of *B.t.* that is mutagenic in mammals; this toxin is not present in *B.t.k.*

biliary — referring to bile, a substance in which many chemicals are eliminated from the body

bioassay — determination of the relative strength of a substance (e.g., drug, insecticide) by comparing its effect on a test organism with that of a standard preparation

biodiversity — variety of life and its processes; includes the variety of living organisms, the genetic differences among them, and the communities and ecosystems in which they occur

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

biomass — total weight, volume, or energy equivalent of organisms in a given area

biota — plants and animals

BIU — acronym for billion international units

B.t.k. — abbreviation for *Bacillus thuringiensis* var. *kurstaki*

C

cancer potency parameter — model-dependent measure of cancer potency (mg/kg/day) over lifetime exposure; often expressed as a q, which is the upper 95 percent confidence limit of the first dose coefficient (q,) from the multistage model

canopy — uppermost layer of foliage in forest

vegetation, formed by the crowns of trees

carcinogen — chemical capable of inducing cancer

carcinoma — malignant tumor

carrier — in commercial formulations of insecticides or control agents, a substance added to the formulation to make it easier to handle or apply

caterpillar — soft-bodied larva of the gypsy moth or other moth, butterfly, or sawfly

cell-mediated response — response originating from materials within the cell, in contrast to a humoral response

cfu — acronym for colony forming units

chironomid — ecologically important group of aquatic insects belonging to the family Chironomidae (order Diptera), often occurring in high densities and diversity, and feeding on a great variety of organic substrates; important prey of most aquatic predators

chitin — hard substance made of a complex carbohydrate (acetyl glucosamine) similar to cellulose; main component in the skin (cuticle) of insects, spiders, and crustaceans

cholinergic — refers to nerve cells that release acetylcholine

cholinesterase — group of enzymes that degrade acetylcholine and similar compounds. Cholinesterases that occur in nerve tissues have a clear function. Other cholinesterases, such as those occurring in red blood cells or plasma, do not have a clear function but are used as indicators of insecticide exposure

chromatography — method of separating chemicals prior to quantitative analysis

chronic exposure — long-term exposure studies often used to determine the carcinogenic potential of chemicals; these studies are usually performed on rats, mice, or dogs and extend over the average lifetime of the species; for example, chronic exposure for a rat is 2 years

chronic toxicity — adverse biologic response, such as mortality or an effect on growth or reproductive success, resulting from repeated or long-term (equal to or greater than 3 months) doses (exposures) of a compound, usually at low concentrations

circadian rhythm — influence of the time of day on the rate of metabolism of foreign compounds, often observed in a given animal species; a variation in the metabolic rate often correlated with variations in endocrine functions, as influenced by the light-dark cycle to which the animal is exposed

cladoceran — small aquatic crustaceans in the order Cladocera; water fleas

coliforms — bacteria that indicate recent fecal contamination of water

colony forming unit (cfu) — index of bacterial levels in a medium such as air or water; a cfu represents a collection of a droplet or particulate from air that contains one or more viable spores or vegetative cells of *B.t.k.*

common logarithm — common logarithm of a number, X, is defined as the number, Y, which when used as the exponent of 10 results in X. Thus, if X = 10^1, then the log of X is Y, which is often written using the notation, log(X) = Y

community — association of potentially interacting plants and/or animals, more or less distinguishable from other such associations, usually defined by the nature of their interaction or the place in which they live

compliance agreement — written agreement between APHIS Plant Protection and Quarantine and a person who grows, handles, or moves regulated articles to comply with APHIS regulations

confounders — term used in discussions of studies regarding human populations (epidemiology studies) to refer to additional risk factors which, if unaccounted for in a study, may lead to erroneous conclusions

congenital — refers to conditions present at birth, regardless of their cause

conidium — asexual spore produced by fungi (pl. conidia)

conjugation — in metabolism, a linkage of one molecule with another; common step in the elimination of many chemicals from the body

conjunctiva — thin mucous membrane that lines the eyelids

conjunctivitis — inflammation of the membrane that lines the eyelids

connected actions — exposure to other chemical and biological agents in addition to exposure to a treatment agent used to control gypsy moth

connective tissue — tissue that binds together and supports the various structures of the body

contaminants — for chemicals, impurities present in a chemical-grade chemical; for biological agents, other agents that may be present in a commercial product

control — maintain or try to maintain a population density of insects or other undesirable animals below the point where injury to man's interests occurs

conspecific — belonging to the same species

cooperative project — management project conducted by a State or Federal agency, under agreement and with financial and technical assistance of the U.S. Department of Agriculture, to control forest diseases and insects such as the gypsy moth

Cooperative State Research, Education, and Extension Service — (CSREES) USDA agency that administers a research grants program, including gypsy moth research; plans cooperative research projects through the State Agriculture Experiment Station System and coordinates information and education activities

cooperator — State or Federal agency that enters into an agreement with the U.S. Department of Agriculture

to conduct a cooperative project

copepod — small marine or freshwater crustaceans in the class Copepoda, exhibiting great diversity in form and life history

corixid — insects in the family Corixidae (order Hemiptera); referred to as true water bugs

corneal opacity — cloudy area on the cornea

corneal ulcer — small area of damaged tissue on the surface of the eye

corticosteroid — anti-inflammatory agent

corrosive effect — effect that causes visible destruction and alteration in tissue at the site of contact

cover type — vegetation, described in terms of its general form or dominant species, comprising the plant community in a given area

critical habitat — area determined by the U.S. Fish and Wildlife Service to be essential to the conservation of threatened or endangered species and that may require special management considerations or protection

crown condition — combination of tree crown density, coloration, leaf-rolling, mortality, or other factors that provide an indication of tree health

crustaceans — organisms such as crabs, lobsters, shrimp, crayfish, wood lice, pill bugs, and water fleas that have hard exoskeletons made of chitin, as do other arthropods

CSREES — acronym for Cooperative State Research, Education, and Extension Service

cumulative effects — effects attributable to exposure(s) that may last for several days to several months, or effects resulting from gypsy moth program activities that are repeated more than once during a year or for several consecutive years

cumulative exposure — exposure that may last for several days to several months or exposures resulting

from program activities that are repeated more than once during a year or for several consecutive years

cytosolic — found in the cytoplasm of a cell

D

dam(s) — female parent(s)

DDVP — abbreviation of the chemical name for dichlorvos—2,2 dichloroethenyl dimethyl ester phosphoric acid—an insecticide contained in some gypsy moth traps

defoliation — noticeable loss of foliage due to feeding by insects, such as gypsy moth caterpillars; light defoliation is normal background defoliation of less than 30 percent, moderate defoliation is 30 to 60 percent, heavy defoliation is greater than 60 percent

defoliation survey — visually examining trees from the ground or the air, to detect defoliation

degradation — breakdown of a compound by physical and chemical or biochemical processes, into basic components with properties different from those of the original compound

degraded — broken-down or destroyed

degrees of freedom — number of data points minus the number of parameters in a model. For example, two points are required to define a straight line. In statistical jargon, using two points to define a straight line is fitting a two-parameter model with zero degrees of freedom

delimiting survey — using pheromone-baited traps to determine the approximate size of an infested area

delineation — a process used in slow the spread to estimate numbers and presence of gypsy moths in an area

delta-endotoxin — proteinaceous toxin in *B.t.k.* that is toxic to gypsy moth larvae

dermal — pertaining to the skin

dermatitis — inflammation of the skin; characterized by redness, swelling, pain, and warmth

detection survey — using pheromone-baited traps to determine whether the gypsy moth is present and where delimiting may be necessary

detritus — fragmented, particulate-organic matter resulting from the decomposition of plant and animal remains

developed forest — privately owned forested residential areas

dichlorvos — another name for DDVP

diflubenzuron — active ingredient of chemical insecticide formulations sold under the trade name Dimilin®; acts as a growth regulator by interfering with chitin synthesis, preventing molting in gypsy moth caterpillars, some other immature insets, and crustaceans

Dimilin® — trade name of diflubenzuron formulations registered for use against the gypsy moth

DiPel — one of the commercial formulations of *B.t.k.*

dipteran — insect belonging to the order Diptera (meaning two wings), which includes flies and mosquitoes

direct effect — reaction of an organism after exposure to a chemical or non-chemical agent that is not medicated through another organism. For example, caterpillars that eat leaves with diflubenzuron on them fail to molt, and die as a result of their direct exposure to this insecticide; the direct effect of an unchecked gypsy moth infestation could be a change in species composition of trees

dislodgeable residues — residue of a chemical or biological agent on foliage as a result of aerial or ground spray applications, which can be removed readily from the foliage by washing, rubbing, or having some other form of direct contact with the treated vegetation

disparlure — synthetic version of the pheromone produced by female gypsy moths to attract males for mating

diuresis — increased urinary excretion

diurnal rhythm — normal changes in the body that occur during the day; most diurnal variations have been shown to be related to eating and sleeping habits

dominant trees — trees with crowns extending above the general level of the canopy and receiving full light from above and from the side

dose — quantity of material that is taken into the body; dosage is usually expressed in amount of substance per unit of animal body weight, often in milligrams of substance per kilogram (mg/kg) of animal body weight, or other appropriate units; in radiology, the quantity of energy, or radiation absorbed

dose-response assessment — description of the relationship between the dose of a chemical and the occurrence or intensity of an effect

draft environmental impact statement — detailed, written statement of effects expected as a result of a major Federal action that is released to the public and other agencies for review and comment, as required under Section 102 (2)(c) of the National Environmental Policy Act

E

EC_{50} — acronym for median effective concentrate

ecology — study of the interrelationships between living organisms and their environment

ecosystem — living organisms interacting with each other and with their physical environment, usually described as an area for which it is meaningful to address these interrelationships

ecosystem management — holistic approach to achieving productive healthy ecosystems by blending social, physical, economic, and biological needs and values

eczema — form of dermatitis associated with swelling and redness of the skin

effect level — dose or concentration of a substance reported to have no harmful (adverse) effects on people or animals.

effector cell — cell stimulated by a nerve cell to effect a certain function. Examples include muscle and sensory cells

egg mass survey — visually examining an area in a systemic manner, either (1) outside the generally infested area, to obtain evidence that gypsy moths are present and reproducing, or (2) in an infested area, to assess the population density

EIS — acronym for environmental impact statement

empirical — refers to an observed, but not necessarily fully understood relationship; in contrast to a hypothesized or theoretical relationship

enantiomer — reference to molecules that are structurally identical except for differences in the three-dimensional configuration

endangered species — Federal designation for any species that is in danger of extinction throughout all or a significant part of its range. The Federal list of endangered species is maintained by the Secretary of the Interior

endemic — something that is always present in a population but not always prevalent or present in high numbers; often applied to diseases or infestations

endospore — thick-walled body containing genetic material that forms inside the vegetative cell of some types of bacteria, including bacillus, under adverse conditions. When conditions improve, the endospore can develop into a vegetative cell

endpoints — components of an ecosystem that indicate its sensitivity to the type of disturbance expected from the gypsy moth or treatments; five endpoints were selected for the ecological risk assessment:

nontarget organisms, forest condition, water quality, microclimate, and soil fertility and productivity

Entomophaga maimaiga — scientific name for a fungus that causes disease in gypsy moth caterpillars

environmental analysis — investigation of alternative actions and their predictable environmental effects through a systemic interdisciplinary approach, which ensures the integrated use of the natural and social sciences and the environmental design arts in planning and in decision making that may have an impact on the human environment

Environmental Assessment — **(EA)** a concise public document that a Federal agency prepares under the National Environmental Protection Act (NEPA) to provide sufficient analysis and evidence for either a finding of no significant impact or preparation of an environmental impact statement

Environmental Impact Statement — (EIS) a detailed public document written by a Federal agency to disclose significant environmental impacts that would result from a planned action and used to make decisions about the action

enzyme — biological catalyst; a protein produced by an organism itself, which enables the splitting (as in digestion) or fusion of other chemicals

Ephemeroptera — order of aquatic insects including mayflies

epidemiology — branch of science that deals with the incidence, distribution, and control of disease in a population

epidermis — outermost layer of the skin

epizootic — occurrence of a disease in animals that is widely prevalent and spreads rapidly

eradication — strategy of eliminating an isolated infestation of the gypsy moth

erythema — name applied to redness of the skin produced by congestion of the capillaries, which may result from a variety of causes

erythrocyte — red blood cell

European strain — strain of the gypsy moth historically found in Western Europe and the original source of the North American population, which has females that do not fly

evaluation — gypsy moth survey to determine the need for treatment or to determine the effectiveness of treatment

exclusion — policy pursued by APHIS to prevent animal and plant pests and diseases, including the gypsy moth, from being introduced into the United States

exotic — refers to all species of plants and animals not naturally occurring, either now or in the past, in an ecosystem of the United States

exposure — skin contact, inhalation, or ingestion of a substance that may have a harmful effect

exposure assessment — process of estimating the extent to which a population will come into contact with a chemical or biological agent

extra risk — risk in the population that can be attributed to exposure to the agent

extrapolation — use of a model to make estimates outside of the observable range

exuviae — cast-off skins or outer coverings of insects and animals that shed skin

F

fecal — relating to feces (solid waste)

fibroma — benign tumor composed mainly of fibrous or fully developed connective tissue

fibrosarcoma — malignant tumor derived from fibroblasts that produce collagen

FIFRA — Federal Insecticide, Fungicide, and Rodenticide Act; establishes procedures for the registration, classification, and regulation of pesticides

final environmental impact statement — detailed, written statement of the analysis of a major Federal action, released to the public as required under sec. 102 (2)(c) of the National Environmental Policy Act

financial assistance — money provided by the Forest Service and APHIS to Federal and State agencies through several pest control or management programs to suppress, eradicate, or slow the spread of the gypsy moth. On Federal lands the cost of gypsy moth projects are paid in full; on State and private lands cost may be shared with State cooperators. See technical assistance for other assistance provided

food chain — feeding sequence used to describe the flow of energy and materials through the system

food web — interconnected food chains in the ecosystem, representing the various paths of energy flow through populations in the community

Foray — one of the commercial formulations of *B.t.k.*

forest — land at least 10 percent occupied by forest trees or formerly having had such tree cover and not currently developed for non-forest use. Lands developed for non-forest use include areas for crops, improved pasture, residential or administrative areas, improved roads of any width, and adjoining road-clearing and power line clearing of any width

forest condition — species composition, tree growth rates and mortality rates, productivity, and degree of insect damage

forest cover type — description based on and named after the tree species that forms a plurality of the basal area in a stand; other tree species may also be part of the stand

Forest Service — lead agency for this environmental impact statement; the largest USDA agency, which conducts research and develops the means to control the gypsy moth in forests; conducts surveys and evaluations on lands managed by other Federal

agencies; helps State and other Federal agencies to conduct detection surveys, evaluation and suppression; to test and transfer technology designed to improve gypsy moth control and reduce damage; and to conduct eradication on Federal or adjacent land, and on non-Federal land for infestations of more than 640 acres

forest type group — grouping of forest cover types for inventory, mapping, or other purposes

forestomach — front or foremost portion of the stomach in animals

formulation — commercial preparation of a chemical including any inert ingredients or contaminants

frank effects — obvious signs of toxicity

Frank Effect Level (FEL) — dose or concentration of a chemical or biological agent that causes gross and immediately observable signs of toxicity

frass — fecal excrement of gypsy moth caterpillars

fumigant — pesticide applied as a liquid or powder which volatilizes to gas; usually applied beneath a tarp, sheet, or other enclosure

fumigation — process of using a fumigant to destroy pests, usually applied under a cover or shelter

FWS — Fish and Wildlife Service, an agency of the U.S. Department of the Interior

G

gavage — placement of a toxic agent directly into the stomach of an animal, using a gastric tube

gene — basic unit of inheritance, by which hereditary characteristics are transmitted from parent to offspring. Genes consist of short lengths of DNA (or RNA in some viruses) that direct the synthesis of protein. These in turn influence the form and function of the organism

generally infested area — (regulated or quarantine area) the area in the eastern United States where the European strain of the gypsy moth is considered to be permanently established; also the area quarantined by APHIS and the States. All life stages are present, and populations are continuous. Population outbreaks occur, and defoliation is common. In 1994, the area extended from Maine to northern North Carolina and west to West Virginia, Ohio, and Michigan

genotoxic — causing direct damage to genetic material, associated with carcinogenicity

genotoxicity — specific adverse effect on the genome (the complement of genes contained in the haploid set of chromosomes) of living cells, which upon the duplication of the effected cells can be expressed as a mutagenic or carcinogenic event because of specific alteration of the molecular structure of the genome

geocorid — big-eyed bug

geometric mean — measure of an average value often applied to numbers for which a log-normal distribution is assumed

gestation — period between conception and birth; in humans, the period known as pregnancy

gram (g) — metric unit of measure for weight or mass

growth regulator — chemical that controls the rate of growth, or interferes with successful growth in an animal; diflubenzuron is a growth regulator for insects and other chitinous animals

guild — group of species with similar modes of existence

Gypchek — trade name for a biological insecticide containing gypsy moth nucleopolyhedrosis virus, which is registered and produced by the Forest Service and APHIS

gypsy moth — all life stages of the Asian and European strains of the insect with the scientific name *Lymantria dispar* (L.), previously *Porthetria dispar* (L.)

H

Haber's Law — in toxicology, the assumption that the concentration or dose, multiplied by the duration of exposure (time) will always have the same effect. This relationship is true for some chemicals and some endpoints but not true for others. Even when true for a particular chemical and effect, it may be true only over certain ranges of exposure

habitat — place or type of site where a plant or animal naturally or normally lives and grows

half-life — time required for the concentration of a chemical to decrease by half of the original concentration (the longer the half-life, the more persistent a chemical is considered to be)

hazard — adverse effects to humans or the environment as a result of exposure to the gypsy moth or treatments; compare risk

hazard assessment — component of a risk assessment that consists of the review and evaluation of toxicological data to identify the nature of the hazards associated with a chemical, and to quantify the relationship between dose and response

hazard identification — process of identifying the array of potential effects that an agent may induce in an exposed population

hazard quotient — ratio of the estimated level of exposure to the risk-reference value or some other index of acceptable exposure; a hazard quotient greater than 1 raises concern

Heinz bodies — dark-staining granules found in red blood cells, which are signs of oxidative damage; formation of Heinz bodies can lead to red cell dysfunction and breakdown of the cell membrane

hemangiosarcoma — malignant tumor formed by proliferation of endothelial and fibroblastic tissue

hematological — pertaining to the blood

hemipteran — insect belonging to the order Hemiptera, including the true bugs

hemoglobin — iron-containing respiratory pigment in red blood cells of vertebrates

herbaceous — relating to plants that have nonwoody stems and die down annually

herbivorous insect — insect that eats plants and plant material; the gypsy moth is an herbivorous insect because it eats leaves

HHERA — acronym for Human Health and Ecological Risk Assessment

histamine — naturally occurring chemical; causes dilation of the capillaries and muscle contraction

histopathology — signs of tissue damage that can be observed only by microscopic examination

homopteran — insect in the order Homoptera, which includes aphids, scale insects, and cicadas

host — living organism that provides subsistence or lodging for another organism

humoral — associated with agents dissolved in the blood or body fluids, in contrast to materials contained in cells (cell-mediated)

hydroxylation — addition of a hydrogen-oxygen or hydroxy (−OH) group to one of the electron rings of a compound. Hydroxylation increases the water solubility of aromatic compounds, particularly when followed by conjugation with other water-soluble compounds in the body, such as sugars or amino acids, hydroxylation greatly facilitates the elimination of the compound in the urine or bile

hymenopteran — any of highly specialized insects in the order Hymenoptera, usually with four membranous wings, the abdomen borne on a slender pedicel and associated with large colonies and complex social organization; includes bees, wasps, ants, ichneumonid flies, sawflies, and gall wasps

hypoactivity — less active than normal

I

immunocompetent — having normal immune function

immunocompromised — having an impaired immune system, such as people with HIV or AIDS

immunodeficient — organism with impaired immune function

in vitro — in glass; a test-tube culture; any laboratory test using living cells taken from an organism

in vivo — in the living organism; in vivo tests are those laboratory experiments carried out on whole animals or human volunteers

indirect effect — reaction of an organism to a change in the environment that is a direct result of exposure to a chemical or non-chemical agent. For example, wasps that prey on caterpillars that eat leaves with diflubenzuron on them could obtain diflubenzuron that the caterpillars ate, thus exposed indirectly to the chemical; the indirect effect of an unchecked gypsy moth infestation could be the change in woodland structure, a direct effect of the gypsy moth

inerts — adjuvants or additives in commercial formulations of gypsy moth control agents that do not cause mortality in the gypsy moth

inert ingredients — additives in insecticide formulations that do not effect the organism targeted but are added for a variety of reasons, such as to stabilize the formulation, to improve its weatherability, or to prevent growth of contaminating microorganisms

infestation — presence of the gypsy moth and an indication of a reproducing population, based on the results of surveys

infested area — isolated infestation or generally infested area

inhalation — act of breathing

innocuous — something that produces no injury; harmless; inoffensive

insecticide — pesticide that kills, debilitates, or controls the growth of insects

instar — stage between molts in the development of the gypsy moth caterpillar and other arthropods

Integrated Pest Management (IPM) — selecting strategies to mange pest-host systems for specific objectives; includes planning, detection, evaluation, monitoring, establishing acceptable damage thresholds, and use of appropriate management practices to prevent or control pest-caused damage and losses

intercept — in a simple linear equation, the value of the dependent variable when the independent variable is zero

interdisciplinary team — team of varied resource specialists with different professional backgrounds who conduct an environmental analysis; members of the interdisciplinary team who prepared this environmental impact statement are listed in chapter 5, Preparers and Contributors

interpolation — use of mathematical models within the range of observations

intraperitoneal — injection into the abdominal cavity

invertebrates — animals without a spinal column, such as insects, spiders, and crustaceans

IPM — acronym for Integrated Pest Management

iritis — inflammation of the iris

irritant effect — reversible effect, compared with a corrosive effect

isolated infestation — defined area infested with the gypsy moth outside the generally infested area; or, a defined area infested with the Asian strain of the gypsy moth within the generally infested area

issue — public concern or significant problem that might occur when the gypsy moth is present or treatments are applied

IU — International Unit

L

land use — type of activity occurring on the land surface, e.g. forestland, farmland, pastureland, etc

landscape — physical features of an area (e.g. slope, aspect, drainage) that affects the characteristics of the plant and animal communities in the ecosystem

Latin Hypercube — stratified sampling technique designed to sample from all portions of a distribution

larva — stage in development between hatching and attaining adult form

larval survey — placing tar paper, burlap, or similar material around the trunks of susceptible trees, to create hiding places for gypsy moth caterpillars so they can be captured and counted

LC_{50} — acronym for lethal concentration$_{50}$

LD_1 — acronym for lethal dose$_1$

LD_{50} — acronym for lethal dose$_{50}$

leaf expansion — percentage of leaf growth from 0 to 100

lentic — water bodies that do not flow (e.g., lakes, ponds)

lepidopteran — insects in the order Lepidopteran, characterized by adults with two pairs of scale-covered wings and coiled sucking-mouthparts, including moth and butterflies

lethal concentration$_{50}$ (LC_{50}) — calculated concentration of a toxicant in air (or water) to which exposure for a specific length of time is expected to cause death in 50 percent of a defined test animal population

lethal dose$_1$ (LD_1) — dose of a chemical or biological agent calculated to cause death in 1 percent of a defined test animal population

lethal dose$_{50}$ (LD_{50}) — dose of a chemical or biological agent calculated to cause death in 50 percent of a defined test animal population

lethargy — decrease in the normal amount of activity

life stage — distinctive period in an insect's life (Nichols 1989); life stages of the gypsy moth are: egg (in an egg mass), larva or caterpillar, pupa, and adult moth

lipophilic — having a tendency to dissolve or partition to fatty substances

LOAEL — acronym for lowest-observed-adverse-effect level

log-normally — a logarithmic function with a normal distribution

lotic — water bodies that flow and have running waters (e.g. streams, rivers)

lowest-observed-adverse-effect level (LOAEL) — lowest measured amount of a chemical that produces significant increases in frequency or severity of adverse effects in an exposed human population

M

macroinvertebrates — invertebrates large enough to be seen with the unaided eye

malignant — cancerous

mammary gland — breast

management practice — specific act, measure, cause of action, or treatment

mass trapping — using pheromone-baited traps to catch all or nearly all the male gypsy moths in an area having low gypsy moth populations

mast — fruit and seeds of trees and other forest vegetation eaten by wildlife; hard-mast includes nuts and seeds (such as acorns, walnuts, hickory nuts, maple

seeds); soft-mast is fruit (such as apples, blackberries, wild grapes)

mating disruption — saturating an area with gypsy moth pheromone to confuse male gypsy moths, thereby preventing them from locating and mating with females

median effective concentration (EC_{50}) — concentration of a substance that results in some effect being exhibited by 50 percent of the test organisms

median lethal concentration — concentration of a toxicant necessary to kill 50 percent of the organisms in a population being tested; usually expressed in parts per million (ppm), milligrams per liter (mg/L), or milligrams per cubic meter (mg/m^3)

median lethal dose — dose necessary to kill 50 percent of the test organisms; usually expressed in milligrams of chemical per kilogram of body weight (mg/kg)

metabolite — compound formed as a result of the metabolism or biochemical change of another compound

metastatic — pertaining to or of the nature of metastasis; the transfer of disease from one organ or part to another not directly connected with it; may be due either to the transfer of pathogenic microorganisms (e.g., bacilli) or to the transfer of cells, as in malignant tumors

methemoglobinemia — rare blood disorder in which there is a deficiency of the enzyme that turns methemoglobin into hemoglobin (methemoglobin differs from hemoglobin in being unable to combine reversibly with oxygen)

mg/cm² — milligrams per square centimeter

mg/kg — milligrams per kilogram

mg/m³ — milligrams per cubic meter

microclimate — climate of the immediate surroundings or habitat, differing from the macroclimate, as a result of the influences of local topography, vegetation and soil

microinvertebrates — invertebrates too small to be seen without magnification

microlepidopterans — general term for the most primitive families of moths whose members usually have the smallest body size among lepidopterans

microorganism — organism so small that a microscope is necessary to see it

microsomal — pertaining to portions of cell preparations commonly associated with the oxidative metabolism of chemicals

mineralization — conversion of an organic substance into an inorganic substance as a result of microbial decomposition

minimal risk level (MRL) — route-specific (oral or inhalation) and duration-specific estimate of an exposure level that is not likely to be associated with adverse effects in the general population, including sensitive subgroups

mixture of concern — mixture on which a risk assessment is being conducted. See sufficient similarity.

molting — process of shedding an old skin and creating a new one, as an insect grows or changes in form

monitor — to observe or check that treatments are carried out as planned, or to determine whether effects of treatments are those that were predicted

Monte Carlo simulation — technique used to simulate systems with probabilistic elements; one or more variable in a Monte Carlo simulation is determined by drawing a random number from a probability distribution (such as the normal or uniform distribution), which describes the natural variation in that variable

most-sensitive effect — adverse effect observed at the lowest dose of a substance—an important concept in risk assessments; if the most-sensitive effect is prevented, no other effects will develop

multiple-chemical sensitivity — syndrome that affects individuals who are extremely sensitive to chemicals at extremely low levels of exposure

mutagenicity — ability of a substance (mutagen) to cause genetic damage, that is, damage to DNA or RNA (mutation); mutations can lead to birth defects, miscarriages, or cancer

N

nabid — damselbug belonging to Order Hemiptera of Class Insecta

NADH — acronym for nicotinamide adenine dinucleotide phosphate; a molecule that is common in all living systems and is necessary for the proper function of many enzymes

nanogram (ng) — one billionth of a gram

National Environmental Policy Act (NEPA) Act of 1969 (42 U.S.C. 4321) — established a national policy that encourages harmony between man and the environment; requires that Federal agencies proposing legislation or a major action use a systemic, interdisciplinary approach to planning and decisionmaking, and prepare a detailed statement that includes the following: the environmental impact of the proposed action, any adverse environmental effects that cannot be avoided, alternatives to the proposed action, the relationship between local short-term uses of man's environment and the maintenance and enhancement of long-term productivity, and any irreversible and irretrievable commitment of resources

National Gypsy Moth Survey — minimal detection survey administered by APHIS in cooperation with the States to detect isolated infestations of the gypsy moth outside the generally infested area

natural landmark — site on the National Registry of Natural Landmarks, administered by the National Park Service, U.S. Department of the Interior, preserved as an outstanding example of plant or animal communities, geological features, scenic grandeur, or other attribute

natural spread — movement of gypsy moths from an infested area: (1) of first instar larvae by wind, (2) of larger larvae by crawling, (3) of adult females of the European strain by crawling, (4) of some adult females of the Asian strain by flying

necropsy — examination of a body after death, usually refers to a gross examination of the major organs

nematodes — elongated cylindrical worms that are parasitic in animals or plants or free-living in soil or water

neotropical migrant — bird that nests in North America but migrates to the Neotropics (region of the New World south of the Tropic of Cancer, includes South America, Central America, southern Mexico, the West Indies, and Caribbean) during winter

NEPA — acronym for National Environmental Policy Act

neuropathy — damage to the peripheral nervous system

ng — nanogram, one billionth of a gram

NIOSH — acronym for the National Institute for Occupational Safety and Health

nm — nanometer, one billionth of a meter

NOAEL — acronym for non-observed-adverse-effect level

NOEL — acronym for no-observed-effect level

no-observed-adverse-effect level (NOAEL) — highest measured amount of a chemical at which no increase in frequency or severity of adverse effects is observed in an exposed human population when compared with a control; effects may be produced, but they are not considered to be adverse

no-observed-effect level (NOEL) — dose of a chemical or biological agent at which there are no biologically or statistically significant effects attributable to treatment

non-insecticidal treatments — gypsy moth treatments that do not involve spraying of insecticides; in this environmental impact statement, they include mass trapping, mating disruption, and the sterile insect technique

non-target organism — any living organism that is not the target of a management practice

normal distribution — theoretical frequency-distribution of variable data generally shaped in a bell-shaped curve

Notice of Intent — announcement that preparation of a new national gypsy moth supplemental environmental impact statement was beginning, which appeared in the April 29, 2004, Federal Register (vol. 69, no. 83, p. 23,492 – 23,493)

NPV — acronym for nucleopolyhedrosis virus

nucleopolyhedrosis virus (NPV) — category of naturally occurring viruses that cause a usually fatal disease, mainly in larvae of moths, butterflies, sawflies, wasps, ants, bees, and others. The nucleopolyhedrosis virus specific to the gypsy moth is the active ingredient in the insecticide Gypchek

nymph — larvae of an insect with incomplete metamorphosis that differs chiefly in size and degree of differentiation from the final adult stage

O

OB — acronym for occlusion bodies

occlusion bodies (OB) — virus particles containing variable numbers of genetic material within one protein envelope

octanol-water partition coefficient (K_{ow}) — equilibrium ratio of the concentrations of a chemical in n-octanol and water, in dilute solution

ocular — pertaining to the eye

odonates — insects in the order Odonata; dragonflies and damselflies

1-day health advisory — drinking water concentration (mg/L) not likely to cause adverse effects in the general population, including sensitive subgroups, after 1 day of exposure

one-storied stand — stand of trees that is characterized by the predominance of trees the same size

ophthalmic — pertaining to the eye, as an ophthalmic solution–a solution of medication intended to be applied to the eye

oral — pertaining to the mouth

oral toxicity — toxicity of a compound when given or taken by mouth, usually expressed as milligrams of chemical per kilogram of body weight of animal (mg/kg)

organoleptic — relating to an objectionable taste or smell

organophosphate — class of insecticides that are toxic to the nervous system

orthopteran — insects in the order Orthoptera, which includes grasshoppers, crickets, locusts, and cockroaches

osteosarcoma — malignant tumor derived from bone tissue

outbreak — cyclic rise in gypsy moth populations when feeding by caterpillars causes widespread moderate-to-heavy defoliation

ovicide — chemical toxic to the eggs of the target animal

P

parasite — organism that lives in, on, or at the expense of another, from which it obtains food, shelter, or other requirements; a parasite is usually smaller than the host and weakens it

parasitoid — parasite that eventually kills its host, for

example, insects that kill life-stages of the gypsy moth

parenteral — any form of injection

partition — in chemistry, the process by which a compound or mixture moves between two or more media

pathogen — an agent, such as a virus or bacterium, that causes disease

pathogenic — causing or capable of causing disease

pathway — in metabolism, a sequence of metabolic reactions

peroxide — molecule that contains two or more oxygen atoms in series, such as —O—O—; these molecules are often involved in the degradation of polymers, including proteins

persistence — characteristic of an insecticide or a compound to remain in the environment as an effective residue; persistence is related to volatility, chemical stability, and degradation

pesticide — substance or mixture of substances that kill insects, rodents, fungi, weeds, or other forms of plant or animal life that are considered to be pests

pH — measure of acidity and alkalinity on a scale from 0 to 14, of which 7 is neutral; lower numbers are acidic, higher numbers are alkaline; numbers vary by a factor of 10, i.e., pH 3 is 10 times more acidic than pH 4

pharmacokinetics — quantitative study of the metabolic processes of absorption, distribution, biotransformation, and elimination of drugs

pheromone — chemical produced and emitted by an animal as a form of communication with other individuals of the same species, for example, the sex attractant given off by the female gypsy moth to attract males for mating

phytoplankton — small algal cells suspended in the water column of water bodies

phytotoxic — toxic or harmful to plants

piloerection — condition in which the hair stands on end

pituitary-adrenal axis — hormonal interaction between the pituitary and the adrenal glands

planktonic — suspended in the water of seas, lakes, rivers, or other water bodies

plasma cholinesterase — another term for pseudo-cholinesterase; the normal physiological role of this cholinesterase is not known, inhibition of this enzyme is considered an index of exposure to many oganophosphate insecticides

plasma — fluid portion of the blood in which particulates are suspended

plasmid — sub-cellular elements in bacteria that contain genetic material for relatively narrow and specific traits; plasmids can be transferred from one microorganism to another of the same species; transfer may also occur between two microorganisms of different species

Plecoptera — order of insects; includes stoneflies

polymer — generic term for a molecule composed of repeating units of less complex molecules; for example, proteins are polymers of amino acids

polyvinyl chloride — nontoxic polymer of vinyl chloride

population — group of gypsy moths that occupy a defined area, separated to some degree from other groups, and are reproducing

population survey — counting egg masses in the generally infested area to determine if suppression treatments are warranted, or using pheromone traps in the transition area to determine if slow-the-spread treatments are warranted

post-treatment evaluation or survey — defoliation, egg mass, or larval survey conducted in a treatment area to evaluate treatment effectiveness

potentiation — action of two or more substances from which one or more (the potentiator) enhances the toxicity of another

ppb — parts per billion; the number of parts of chemical substance per billion parts of the substrate in question

ppm — parts per million; the number of parts of chemical substance per million parts of the substrate in question

predator — animal that obtains the energy it needs to live and grow by eating animals of other species, for example, some mice are predators of the gypsy moth

probit analysis — analysis technique that relates doses to measures of standard deviation away from the 50 percent response level, using the cumulative normal distribution

programmatic — broad or general rather than site specific

proposed species — any species of fish, wildlife, or plant that is proposed in the Federal Register for listing as a threatened or endangered species under the Endangered Species Act

proteinaceous — consisting or composed of proteins

proteolytic enzymes — enzymes that breakdown proteins

prototoxins — proteins that can be converted to toxins

pruritis — itching; an unpleasant skin sensation that provokes the desire to rub or scratch

pseudocholinesterase — term for cholinesterase found in the plasma; the normal physiological role of this cholinesterase is not known; inhibition of this enzyme is considered an index of exposure to many organophosphate insecticides

public involvement — actions taken by the Forest Service and APHIS to involve the various individuals, groups, and organizations who are interested in or may be affected by this environmental impact statement and the decision that may result

pupa — developmental stage of gypsy moth or any lepidoptera, between the caterpillar and adult moth stages, during which the insect undergoes major structural changes

Q

quarantine — designating an area as generally infested, so as to regulate the movement of articles (such as outdoor household articles, logs, and nursery stock) and prevent artificial spread of gypsy moth life-stages to uninfested areas of the United States

R

racemic mixture — 50:50 blend of a (+) enantiomer and (−) enantiomer

recreational forest — publicly owned forest used predominantly for hiking, hunting, camping, day-use, and sightseeing

reference concentration — concentration in air (mg/m³) not likely to be associated with adverse effects over lifetime-exposure, in the general population, including sensitive subgroups

reference dose (RfD) — oral dose (mg/kg/day) not likely to be associated with adverse effects over lifetime exposure in the general population, including sensitive subgroups

regeneration — renewal of a tree or stand of trees; restocking of an area

regulatory activities — activities conducted by APHIS and the States to prevent the artificial spread of the gypsy moth from the regulated area to the uninfested area; activities include inspection and treatment of regulated articles on which the gypsy moth commonly deposits egg masses. See quarantine

renal — pertaining to the kidneys

reproductive effects — adverse effects on the reproductive system that may result from exposure to a chemical or biological agent. The toxicity of the agent may be directed to the reproductive organs or the related endocrine system. The manifestations of these effects may be noted as alternatives in sexual behavior, fertility, pregnancy outcomes, or modification in other functions dependent on the integrity of the reproductive system

residue — quantity of insecticide and its metabolites remaining on and in vegetation, soil, or water

resistance — ability of a population or ecosystem to absorb an impact without significant change from normal fluctuations; for plants and animals, the ability to withstand adverse environmental conditions and/or exposure to toxic chemicals or disease

RfD — acronym for reference dose

rhinitis — inflammation of the mucous membranes of the nose

riparian — pertaining to, living in, or situation on, the banks of rivers and streams (Lincoln and Boxshall 1987)

risk — likelihood that adverse effects will occur; compare hazard

risk assessment — evaluation of the likelihood that adverse effects may occur in humans or the environment as a result of exposure to one or more stressors, such as the gypsy moth and treatments

risk characterization — process of estimating the incidence of a healthy effect in a human population under the different conditions of exposure described in the exposure assessment

risk comparison — the practice of comparing one risk to another in order to promote a better understanding of the consequences of different treatment options as well as the consequences of no treatment

risk reference-value (RRV) — generic term used as

an estimate of dose that is not likely to induce adverse health effects in humans under specific conditions of exposure such as duration and route

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

RRV — acronym for risk reference value

S

safety factor — factor used to give a margin-of-error to the screening index in the Ecological Risk Assessment; safety factors are selected based on the amount of error likely in estimating toxicological benchmark values or concentrations of a toxicant in the environment

salvage — cutting and removing dead, dying, or deteriorating trees before they lose their value as timber

sarcoma — tumor made up of a substance like embryonic connective tissue; often highly malignant

scientific notation — the method of expressing quantities as the product of a number between 1 and 10, multiplied by 10 raised to some power. For example, in scientific notation,
1 kg = 1,000 g [is expressed as] 1 kg = 1×10^3 g; 1 mg = 0.001 [is expressed as] 1 mg = 1×10^{-3} g

scission — in metabolism, breaking or cleavage of part of a molecule

scoping — open process, including public notification and participation, by which an agency identifies significant environmental issues and determines the extent of analysis needed to make an informed decision on a proposed action

screening index — index used to determine whether a species exposed to a toxic agent is at risk. The screening index is a conservative estimate of species at risk. It is more likely to indicate that a species is at risk when it actually may not be than to miss species that are at risk

secondary organism — pathogens or insects that attack trees already weakened by defoliation and that sometimes cause death of the trees

SEIS — acronym for Supplemental Environmental Impact Statement

sensitive subgroup — subpopulation that is much more sensitive than the general public to certain agents in the environment

septicemia — occurrence of pathogens or pathogenic toxins in the blood or other body fluids

serotype — classification of a microorganism based on occurrence of antigens in the cell

silviculture — practice of applying treatments to forest stands, to maintain and enhance them for any purpose (Smith 1986); silvicultural treatments may also be applied to forested areas in urban and suburban areas

slow the spread — strategy being pilot-tested on a large-scale to determine its biological effectiveness and economic efficiency in slowing the gypsy moth's natural spread from areas where it is already established or is a permanent resident by keeping low-level populations from increasing

species composition — assemblage of species inhabiting a defined area

species diversity — ecological concept that incorporates both the number of species in a given area and the number of individuals per species

species richness — number of species in a local area, region, or community

species-to-species extrapolation — method involving the use of exposure data on one species (usually an experimental mammal) to estimate the effects of exposure in another species (usually humans)

squamous-cell papillomas — type of benign tumor

stand — contiguous group of trees sufficiently uniform in species composition, age, and condition to be distinguishable as a unit

stand composition — variety of vegetation species in a stand

stand growth — increases in wood, dry matter, or biomass with a stand

stand structure — combination of species, ages, sizes, and numbers of trees that describe a stand

standard deviation — expression of the variability in a sample or population

standard-normal distribution — normal distribution with a mean of zero and a standard deviation of one

sterile insect technique — gypsy moth treatment that reduces the chance of fertile female gypsy moths mating with fertile males and producing fertile eggs, by the release of large numbers of (1) male pupae sterilized by radiation, (2) male pupae irradiated but not sterilized, or (3) eggs from mating of irradiated males with non-irradiated females

stewardship and stewardship incentives programs — cooperative programs between the Forest Service and States, to provide financial and technical assistance for silvicultural planning on non-Federal forested areas for private landowners

strain — group within a species that differs physiologically rather than in form or structure

strategy — planned actions with specific objectives; the strategies of eradication, suppression, and slow the spread make-up the alternatives examined in this environmental impact statement

Streptococcus (**pl.** ***Streptococci***) — genus of bacteria, which—depending on its classification—may be associated with infections in humans

stressor — an agent, such as an insecticide or the gypsy moth, that causes stress to an ecosystem

subcanopy — cover of branches and foliage formed collectively by trees and other woody growth that is below the principal canopy

subchronic exposure — exposure studies that can last for different periods of time, but 90 days is the most common duration; the subchronic exposure study is usually performed in two species (rat and dog) by the route of intended use or exposure

subchronic reference dose — oral dose (mg/kg/day) not likely to be associated with adverse effects over a less-than-lifetime exposure, in the general population, including sensitive subgroups

subchronic toxicity — adverse biologic response of an organism, such as mortality or an effect on growth or reproductive success, resulting from repeated or short-term (3 month) doses (exposures) of a compound, usually at low concentrations

subconjunctival — refers to the area beneath the membrane that lines the eyelids and eyeball

subcutaneous — just below the skin

subdominant trees — trees with crowns below the general level of the canopy and that receive little or no direct light from above; trees whose crowns make up the subcanopy (Smith 1986)

substrate — with reference to enzymes, the chemical that the enzyme acts upon

succession — natural and gradual replacement of one community of plants by another

succinylcholine — neuromuscular blocking agent

sufficient similarity — as applied to chemical mixtures, whether or not the data on one or more samples of a complex and variable mixture can or should be used for dose-response assessments for all such mixtures

sulfhemoglobinemia — presence of abnormal pigments, other than methemoglobin, in red blood cells

Supplemental Environmental Impact Statement — a document that is written to provide a supplement to the original Environmental Impact Statement

suppression — strategy of reducing outbreak populations of the gypsy moth in areas where it is already established, or is a permanent resident, to prevent or minimize damage to resources

survey — see defoliation survey, delimiting survey, detection survey, egg mass survey, larval survey, National Gypsy Moth Survey, population survey, post-treatment survey, and transition area survey

susceptible plants — plants with leaves the gypsy moth will eat

synapse — space between two nerve cells or a nerve cell and an effector cell such as muscle

synergism — action of two or more substances to achieve an effect of which each is individually incapable; synergistic effects may be greater or less than the sum of effects of the substances in question

synergistic effect — situation in which the combined effects of two chemicals are much greater than the sum of the effect of each given agent alone

systemic — entering and then distributing throughout the body of an organism

systemic effects — effects that require absorption of a toxic agent at an entry point and distribution to a distant site at which effects are produced

systemic toxicity — effects that require absorption and distribution of a toxic agent to a site distant from its entry point at which point effects are produced; systemic effects are the obverse of local effects

T

technical assistance — any of a whole range of direct and indirect help that USDA provides to Federal and State cooperators, short of providing monetary funds; this assistance includes but is not limited to providing training, providing assistance in preparing environmental documents, work and safety plans, contracts, and monitoring plans, and providing assistance on site during the conduct and evaluation of gypsy moth projects

technology transfer — disseminating research results and adapting innovations so government and private parties can use them

1-day health advisory — drinking water concentration (mg/L) not likely to cause adverse effects in the general population, including sensitive subgroups, after 1 day of exposure

teratogenic — relating to or causing developmental malformations

teratology — study of malformations induced during development from conception to birth

thinning from below — silvicultural technique of removing the subdominant trees in a forest stand, leaving the dominant trees more or less evenly distributed over the stand

threatened species — Federal designation for any species that is likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range (the Federal list of threatened species is maintained by the Secretary of the Interior)

threshold — maximum dose or concentration level of a chemical or biological agent that will not cause an effect in the organism

threshold-limit value — air concentration, in milligrams per cubic meter (mg/m^3), not likely to cause adverse effects in exposed workers, over a normal period of work

Thuricide — one of the commercial formulations of *B.t.k.*

toxic — poisonous to organisms

toxicant — poisonous substance such as the active ingredient in pesticide formulations that can injure or kill plants, animals, or microorganisms

toxicity — capacity of a poison to cause adverse effects

toxicological benchmark value (or benchmark value) — values determined for any of a number

of toxicological tests, such as lethal dose $_{50}$, lethal concentration $_{50}$, no-observed-adverse-effect level, lowest-observed-adverse-effect level

toxicology — science that deals with poisons and their effects and problems involved (such as clinical, industrial, or legal)

toxins — chemicals that may cause toxic effects, often used when referring to naturally occurring toxic agents, especially proteins

transition area — area between the uninfested area and generally infested area; populations are discontinuous, consist mostly of adult male moths, and occasionally other life stages; population outbreaks do not occur, and defoliation is uncommon

transition area survey — monitoring gypsy moths in the transition area to provide date that support the decision to quarantine an area or to take other management action

treatment threshold — population level reached by an insect pest that indicates treatment is necessary to prevent unacceptable damage to other resources

triangular distribution — theoretical frequency-distribution shaped like a triangle and described by a minimum, maximum, and likeliest values

trichopteran — insects in the order Trichoptera, in which the adults are terrestrial and immature life stages are almost exclusively aquatic in freshwater; caddisflies

trophic levels — feeding levels—for example, primary producer, herbivore, and first-level carnivore

U

uncertainty factor — factor used in deriving the risk-reference values and similar values from experimental data; uncertainty factors are intended to account for variation in sensitivity among people, the uncertainty in extrapolating animal data to humans, and other sources of uncertainty; common uncertainty factors are 10, 100, and 1,000

understory — vegetation layer below the canopy of other plants, formed by shade-tolerant trees and low shrubs, grasses, and other herbaceous plants

uninfested area — area outside the generally infested area and ahead of the transition area; adult male moths are occasionally found, other life stages are rarely found; no populations are found, and no outbreaks occur

uniform distribution — theoretical frequency-distribution described by a minimum and a maximum value; all values in the uniform distribution have an equal probability of occurrence

Urban and Community Forestry Program — cooperative program between the USDA Forest Service and States to provide financial and technical assistance to municipalities, school districts, communities, and nonprofit organizations for managing trees on non-Federal lands in urban environments

urban forest — forested areas in cities, towns, and communities

urinalysis — testing of urine samples to determine whether toxic or other physical effects have occurred in an organism

urticaria — skin condition marked by the development of wheals

USDA — acronym for U.S. Department of Agriculture

U.S. EPA — acronym for U.S. Environmental Protection Agency

V

vehicle — substance (usually a liquid) used as a medium for suspending or dissolving the active ingredient; commonly used vehicles include water, acetone, and corn oil

vertebrates — animals with a spinal column, such as mammals, fish, birds, amphibians, and reptiles

volatile — referring to compounds or substances that have a tendency to vaporize; material that will evaporate quickly

volatility — tendency of a substance to evaporate at normal temperatures and pressures

vulnerability — likelihood that a tree or plant will die if defoliated

W

watershed — area of land with a characteristic drainage network that contributes to the same surface flow

wheal — smooth, slightly elevated area on the body surface, which is more red or more pale than the surrounding skin; often accompanied by severe itching and usually changing size or shape or disappearing within a few hours; the typical lesion of urticaria, the dermal evidence of an allergic reaction (allergy), and in sensitive persons may be provoked by mechanical irritation of the skin; also called a hive

X

xenobiotic — chemical that does not naturally occur in an organism; term is often applied generically to all synthetic or man-made chemicals

Z

zooplankton — animals that are dependent on movement of water or air for their position or distribution

Chapter 8
References

Figure 8-1. Civilian Conservation Corps workers scouted for gypsy moths.

Chapter 8 References

Figure

References listed are cited in Chapters 1-4 and Appendixes A-E.

A

Aber, R.; DeMelfi, T.; Gill, T.; Healey, B.; McCarthy, M.A. 1982. Rash illnesses associated with gypsy moth caterpillars, Pennsylvania. Morbidity Mortality Weekly Report 31(13): 169-170.

Ables, J.R.; West, R.P.; Shepard, M. 1975. Response of the house fly and its parasitoids to Dimilin© (TH-6040). Journal of Economic Entomology 68(5): 622-624.

Addison, J.A. 1993. Persistence and non-target effects of *Bacillus thuringiensis* in soil: a review. Canadian Journal of Forest Research 23: 2329-2342.

Addison, J.A. 1996. Safety testing of tebufenozide, a new molt-inducing insecticide, for effects on non-target soil invertebrates. Ecotoxicological and Environmental Safety 33(1): 55-61.

Ali, A.; Mulla, M.S. 1978a. Effects of chironomid larvicides and diflubenzuron on non-target invertebrates in residential-recreational lakes. Environmental Entomology 7(2): 21-27.

Ali, A.; Mulla, M.S. 1978b. Impact of the insect growth regulator diflubenzuron on invertebrates in a residential-recreational lake. Archives of Environmental Contamination and Toxicology 7: 483-491.

Ali, A.; Nigg, H.N.; Stamper, J.H.; Kok-Yokomi, M.L.; Weaver, M. 1988. Diflubenzuron application to citrus and its impact on invertebrates in an adjacent pond. Bulletin of Environmental Contamination and Toxicology 41(5): 781-790.

Allen, D.; Bowersox, T. 1989. Regeneration in oak stands following gypsy moth defoliations. In: Rink, George; Budelsky, Carl A., editors. Proceedings: Seventh Central Hardwood Forest Conference; 1989 March 5-8; Carbondale, IL. Gen. Tech. Rep. NC-132. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 67-73.

Anderson, J.F.; Furniss, W. E. 1983. Epidemic of urticaria associated with first-instar larvae of the gypsy moth (Lepidoptera: Lymantriidae). Journal of Medical Entomology 20(2): 146-150.

Andreadis, T.G.; Weseloh, R.M. 1990. Discovery of *Entomophaga maimaiga* in North American gypsy moth, *Lymantria dispar*. Proceedings of the National Academy of Science 87: 2461-2465.

Apperson, C.; Schaefer, C.; Colwell, A. 1977. Effects of diflubenzuron on *Chaoborus astictopus* Dyar and Shannon (Diptera: Chaoboridae) and non-target organisms, and persistence of diflubenzuron in lentic habitats. (Unpublished study received Jul 31, 1978; prepared by North Carolina (California), Mosquito Abatement District; submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL: 234511-AC). MRID No.00099897.

Atkins, E.L.; Greywood-Hale, E.A.; and Macdonald, R.L. 1974. Effect of pesticides on agriculture; 1974 Annual Report: Project No. 1499. (Unpublished study received October 21, 1976 under 6F1696; prepared by The University of California at Riverside Agricultural Experimental Station, Department of Entomology, Citrus Research Center. Submitted by E.I. duPont de Nemours & Company, Inc., Wilmington, Delaware, CDL: 095326-K). MRID No.00040601. Summarized in U.S. EPA 1997a.

ATSDR (Agency for Toxic Substances and Disease Registry) 1993. Cholinesterase-inhibiting pesticide toxicity. Case studies in environmental medicine 22. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

B

Barber, K.N.; Kaupp, W.J.; Holmes, S.B. 1993. Specificity testing of the nuclear polyhedrosis virus of the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae). The Canadian Entomologist. 125: 1055-1066.

Bardwell, C.J.; Averill, A.L. 1996. Effectiveness of larval defenses against spider predation in cranberry ecosystems. Environmental Entomology 25(5): 1083-1091.

Beavers, J.; Ross, T.; Jaber, M. 1993a. RH-5992 Technical: A one generation reproduction study with the Mallard (*Anas platyrhynchos*): Lab Project Number 129-148: 90RC-0222: 91P-222. Unpublished study prepared by Wildlife International, Ltd. MRID No. 42991503.

Beavers, J.; Ross, T.; Jaber, M. 1993b. RH-5992 Technical: A one-generation reproduction study with the Bobwhite (*Colinus virginianus*): Lab Project Number: 129-147: 90RC-0267: HWA 417-481. Unpublished study prepared by Wildlife International, Ltd. MRID No.42991501.

Bell, J.L.; Whitmore, R.C. 1997a. Eastern towhee numbers increase following defoliation by gypsy moths.Auk. 114(4): 708-716.

Bell, J.L.; Whitmore, R.C. 1997b. Bird populations and habitat in *Bacillus thuringiensis* and Dimilin® treated and untreated areas of hardwood forest. American Midland Naturalist. 137(2): 239-250.

Bellinger, R.G.; Ravlin, F.W.; McManus, M.L. 1988. Host plant species and parasitism of gypsy moth (Lepidoptera: Lymantriidae) egg masses by *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae). Environmental Entomology 17(6): 936-940.

Berry, R.E.; Noldenke, A.F.; Miller, J.C.; Wernz, J.G. 1993. Toxicity of diflubenzuron in larvae of gypsy moth (Lepidoptera: Lymantriidae); effects of host plant. Journal of Economic Entomology 86: 809-814.

Bess, H.A.; Spurr, S.H.; Littlefield, E.W. 1947. Forest site conditions and the gypsy moth. Harvard Forest Bulletin 22, Petersham, MA.

Bettancourt, M. 1992. RH-5992 Technical: Toxicity to Fathead Minnow (*Pimephales promelas*) embryos and larvae: Final Report; Lab Project Number 91-9-3923: 86.0291.6133.120: 90RC-0212. Unpublished study prepared by Springbo. MRID No. 42436242.

Biddinger, D.J.; Hull, L.A. 1995. Effects of several types of insecticides on the mite predator *Stethorus punctum* (Coleptera: Coccinellidae), including insect growth regulators and abamectin. Journal of Economic Entomology. 88(2): 358-366.

Biddinger, D.J.; Hull, L.A.; Rajotte, E.G. 1998. Stage specificity of various insecticides to tufted apple bud moth larvae (Lepidoptera: Cidae). Journal of Economic Entomology 91(1): 200-208.

Blumenthal, E.M.; Fusco, R.A.; Readon, R.C. 1981. Parasite augmentation. In: Doane, Charles C.; McManus, Michael L., editors. The gypsy moth: research toward integrated pest management. Technical Bulletin 1584. Washington, DC, U.S. Department of Agriculture: 402-408.

Bogenschutz, H.; Maier, K.; Trzebitzky, C. 1989. Gypsy moth outbreak and control in southwest Germany, 1984-86. In: Wallner, William E.; McManus, Katherine A., technical coordinators. Proceedings, Lymantriidae: a comparison of features of new and old world tussock moths; 1988 June 26-July 1; New Haven, CT. Gen. Tech. Rep. NE-123. Broomall, PA (now Radnor, PA): U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station: 89-99.

Boyle, T.P.; Farichild, J.F.; Robinson-Wilson, E.F. 1996. Ecological restructuring in experimental aquatic mesocosms due to the application of diflubenzuron. Environmental Toxicology and Chemistry 15: 1806-1814.

Broadbent, A.B.; Pree, D.J. 1984. Effects of diflubenzuron and BAY SIR 8514 on beneficial insects associated with peach. Environmental Entomology 13(1):133-136.

Brooks, R.T.; Smith, H.R.; Healy, W.M. 1998. Small-mammal abundance at three elevations on a mountain in central Vermont, USA: a sixteen-year record. Forest Ecology and Management 110(1-3): 181-193.

Brown, J.J. 1996. The compatibility of tebufenozide with a laboratory lepidopteran host-hymenopteran parasitoid population. Biol. Control 6(1): 96-104.

Brown, M.W. 1984. Literature review of *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae), an egg parasite of *Lymantria dispar* (Lepidoptera: Lymantriidae). Entomophaga 29 (3): 249-265.

Brown, M.W.; Cameron, E. A. 1979. Effects of disparlure and egg mass size on parasitism by the gypsy moth egg parasite. *Ooencyrtus kuwanai*. Environmental Entomology 8(2): 77-80.

Brown, M.W.; Respicio, N.C. 1981. Effect of diflubenzuron on the gypsy moth egg parasite *Ooencyrtus kuwanai* (Hymenoptera: Encyrtidae). Melsheimer Entomology Series 31: 1-7.

Buckner, C.H.; McLeod, B.B.; Kingsbury, P.D. 1975. The effect of an experimental application of Dimilin® (4) upon selected forest fauna. Rep. CC-X-97. Ottawa, ON: Environment Canada, Forestry Service, Chemical Control Research Institute.

Bull, D.L.; Coleman, R.J. 1985. Effects of Pesticides on *Trichogramma* spp. Southwestern Entomologist Supplement 0(8): 156-168.

Burrill, E.A.; Worrall, J.J.; Wargo, P.M.; Stehman, S.V. 1999. Effects of defoliation and cutting in eastern oak forests on *Armillaria* spp. and a competitor, *Megacollybia platyphylla*. Canadian Journal of Forest Research 29(3): 347-355.

Butler, L. 1993. Dimilin® impact on Lepidoptera and other canopy arthropods: preliminary results. In: Fosbroke, Sandra L.C.; Gottschalk, Kurt W., eds. Proceedings, U.S. Department of Agriculture Interagency Gypsy Moth Research Form; 1993 January 19-22; Annapolis, MD. Gen. Tech. Rep. NE-179. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station: 28.

Butler, L.; Strazanac, J. 2000. Occurrence of Lepidoptera on selected host trees in two central Appalachian National Forests. Annals of the Entomological Society of America 93(3): 500-511.

Butler, L.; Chrislip, G.A.; Kondo, V.A.; Townsend, E.C. 1997a. Effect of diflubenzuron on non-target canopy arthropods in closed, deciduous watersheds in a central Appalachian forest. Journal of Economic Entomology 90(3): 784-794.

Butler, L.; Kondo, V.; Blue, D. 1997b. Effects of tebufenozide (RH-5992) for gypsy moth (Lepidoptera: Lymantriidae) suppression on non-target canopy arthropods. Environmental Entomology 26 (5): 1009-1015.

C

Cameron, E. A. 1981. On the persistence of disparlure in the human body. Journal of Chemical Ecology 7(2): 313-317.

Cameron, E. A. 1983. Apparent long-term bodily contamination by disparlure, the gypsy moth (*Lymantria dispar*) attractant. Journal of Chemical Ecology 9(1): 33-37.

Campbell, C.A. 1974a. Survival of reptiles and amphibians in urban environments. In: Proceedings of symposium: Wildlife in an urban environment. Planning and Resource Development Series No. 28, Holdsworth National Resources Center: 61-66.

Campbell, R.W. 1974b. The gypsy moth and its natural enemies. Agricultural Information Bulletin. 381. Washington, DC: U.S. Department of Agriculture, Forest Service.

Campbell, R.W. 1979. Gypsy moth: forest influence. Agriculture Information Bulletin 423. Washington, DC: U.S. Department of Agriculture, Forest Service.

Campbell, R.W.; Sloan, R.J. 1976. Influence of behavioral evolution of gypsy moth pupal survival in sparse populations. Environmental Entomology 5(6): 1211-1217.

Campbell, R.W.; Sloan, R.J. 1977a. Forest stand response to defoliation by the gypsy moth. Society of American Foresters, Forest Science Monograph 19.

Campbell, R.W.; Sloan, R. J. 1977b. Natural regulation of innocuous gypsy moth populations. Environmental Entomology 6(2): 315-322.

Campbell, R.W.; Sloan, R.J. 1977c. Release of gypsy moth populations from innocuous levels. Environmental Entomology 6(2): 323-330.

Campbell, R.W.; Valentine, H.T. 1971. Tree condition and morality following defoliation by the gypsy moth. Research Paper NE-236. Broomall, PA (Now Radnor, PA): U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station.

Campbell, R.W.; Hubbard, D.L.; Sloan, R.J. 1975. Location of gypsy moth pupae and subsequent pupal survival in sparse, stable populations. Environmental Entomology 4(4): 596-600.

Campbell, R.W.; Sloan, R.J.; Biazak, C.E. 1977. Sources of morality among late instar gypsy moth larvae in sparse populations. Environmental Entomology 6(6): 865-871.

Cannon, G.; Krize, J. 1976. TH-6040 Egg-to-egg reproduction study in Fathead Minnows: Laboratory No. 5E-6094. (Unpublished study received July 19, 1976 under 148-1262; prepared by Cannon Laboratories, Inc., submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL:096209-I; 225306). MRID No. 00099755.

Carter, J.L.; Schweitzer, D.F.; Reardon, R. 1995. The effects of two applications of *Bacillus thuringiensis* on non-target Lepidoptera. Final report. Hamden, CT: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station.

Centa, A.; Camera, G.; Zucchinetti, P.; DiPetro, P. 1985. Methemoglobinemia in the newborn and nursing infant: genetic and acquired forms (Italian). Pathologica 77(1052): 659-655.

Chandel, R.S.; Gupta, P.R. 1992. Toxicity of diflubenzuron and penfluron to immature stages of *Apis cerana indica* and *Apis mellifera.* Apidologie. 23(5): 465-473.

Clement, R.C.; Nisbet, I.C. 1972. The suburban woodland: trees and insects in the human environment. Audubon Conservation. Rep. 2. New York, NY: National Audubon Society.

Cochran, M.; Poling, P. 1995. Chapter 9. Pollinating insects – Honey bees: 77-80. In: Reardon, R.C., ed. Effects of Diflubenzuron on Non-target Organisms in Broadleaf Forested Watersheds in the Northeast. USDA National Center of Forest Health Management. FHM-CN-05-95. November 1995.

Collins, S. 1961. Benefits in understory from canopy defoliation by gypsy moth larvae. Ecology 42(4): 836-838.

Colwell, A.E.; Schaefer, C.H. 1980. Diets of *Ictalurus nebulosus* and *Poxomis nigromaculatus* altered by diflubenzuron. Canadian Journal of Fisheries and Aquatic Science 37: 632-639.

Cook, S.P.; Smith, H.R.; Hain, F.P.; Hastings, F.L. 1995. Predation of gypsy moth (Lepidoptera: Lymantriidae) pupae by invertebrates at low small mammal population densities. Environmental Entomology 24(5): 1234-1238.

Cooper, R.J. 1988. Dietary relationships among insectivorous birds of an eastern deciduous forest. Morgantown: Ph.D. thesis, West Virginia University.

Cooper, R.J.; Dodge, K.M.; Martinat, P.J.; Donahoe, S.B.; Whitmore, R.C. 1990. Effect of diflubenzuron application on eastern deciduous forest birds. Journal of Wildlife Management 54(3): 486-493.

Corbett, E.S.; Lynch, J.A. 1987. The gypsy moth—does it affect soil and water resources? In: Fosbroke, Sandra; Hicks, Ray R., Jr., eds. Proceedings of the workshop: Coping with the gypsy moth in the new frontier; 1987 August 4-6; Morgantown, WV. West Virginia University Books: 39-46.

Council on Environmental Quality. 1992. Regulations for implementing the procedural provisions of the National Environmental Policy Act. 40 CFR Parts 1500-1508. Washington, DC.

D

Das Gupta, A.; Vaidya, M.S.; Bapat, J.P.; Pavri, R.S.; Baxi, A.J.; Advani, S.H. 1980. Associated red cell enzyme deficiencies and their significance in a case of congenital enzyomopenic methemoglobinemia. Acta Haematol. (Basel) 64(5): 285-288.

Davidson, C.B.; Gottschalk, K.W.; Johnson, J.E. 1999. Tree mortality following defoliation by the European gypsy moth (*Lymantria dispar* L.) in the United States: a review. Forest-Science 45(1): 74-84.

De Clercq, P.; De Cock, A.; Tirry, L.; Vinuela, E.; Degheele, D. 1995. Toxicity of diflubenzuron and pyriproxyfen to the predatory bug *Podisus maculiventris*. Entomologia Experimentalis et Applicata 74(1): 17-22.

DeGraaf, R.M. 1987. Breeding birds and gypsy moth defoliation: Short-term responses of species and guilds. Wildlife Society Bulletin 15: 217-221.

DeGraaf, R.M.; Holland, D.G. 1978. Response of breeding birds to gypsy moth defoliation of an upland oak forest. Trans Northeast Section Wildlife Society 35: 105-119.

Delbeke, F.; Vercruysse, P.; Tirry, L.; De Clercq, P.; Degheele, D. 1997. Toxicity of diflubenzuron, pyriproxyfen, imidacloprid and diafenthiuron to the predatory bug *Orius laevigatus* (Het.: Anthocoridae). Entomophaga 42: 349-358.

Dhadialla, T.S.; Carlson, G.R.; Le, D.P. 1998. New Insecticides with ecdysteroidal and juvenile hormone activity. Annual Review of Entomology 43: 545-569.

Dirr, M.A. 1990. Manual of woody landscape plants: their identification, ornamental characteristics, culture, propagation and uses. 4th ed. Champaign, IL: Stipes Publishing Co.

Doane, C.C. 1970. Primary pathogens and their role in the development of an epizootic in the gypsy moth. Journal of Invertebrate Pathology 15: 31-33.

Doane, C.C. 1971. A high rate of parasitization of *Brachymeria intermedia* (Hymenoptera: Chalcididae) on the gypsy moth. Annals of the Entomological Society of America 64(3): 753-754.

Doane, C.C.; McManus, M.L., eds. 1981. The gypsy moth: research toward intergrated pest management. Tech. Bull. 1584. Washington, DC: U.S. Department of Agriculture.

Downey, D.M. 1991. Do gypsy moths impact stream water chemistry? Appalachian Gypsy Moth Integrated Pest Management Demonstrations Project News 4(9): 1-3.

Downey, D.M.; Armstrong, James D.; Bennett, Kevin H.; French, Christopher R.; Graul, Timothy W. 1994. Impact of watershed defoliation by gypsy moths: water chemistry changes in low ANC head streams. Harrisonburg, VA: James Madison University, Department of Chemistry.

Dubois, N.R. 1991. Insect and foliage development. In: Reardon, Richard, Aerial spraying for gypsy moth control: a handbook of technology. NA-TP-20 [Radnor, PA]: U.S. Department of Agriculture, Forest Service, Northeastern Area and Northeastern Forest Experiment Station: 108-111.

Dubois, N.R.; Reardon, R.C.; Kolodny-Hirsh, D.M. 1988. Field efficacy of the ND-12 strain of *Bacillus thuringiensis* against gypsy moth (Lepidoptera: Lymantriidae). Journal of Economic Entomology 81(6): 1672-1677.

Dunlap, T.R. 1980. The gypsy moth: a study in science and public policy. Journal of Forest History 24(3): 116-126.

Dwyer, J. F.; McPherson, E. G.; Schroeder H. W.; Rowntree, R. A. 1992. Assessing the benefits and costs of the urban forests. Journal of Arboriculture 18: 227-234.

E

Eagle, T.R., Jr. 1993. The effects of gypsy moth defoliation on soil water nutrient concentration in two forest cover types in West Virginia. Morgantown: West Virginia University; Masters thesis: 10-16, 72-81, 147-148.

Ehrenfeld, J.G. 1980. Understory response to canopy gaps of varying size in a mature oak forest. Bulletin of the Torrey Botanical Club 107(2): 29-41.

Elkinton, J.S.; Liebhold, A.M. 1990. Population dyanmics of gypsy moth in North America. Annual Review of Entomology 35: 571-596.

Elkinton, J.S.; Gould, J.R.; Liebhold, A.M.; Smith, H.R.; Wallner, W.E. 1989. Are gypsy moth populations in North America regulated at low density? In: Wallner, William E.; McManus, Katherine A., technical coordinators. Proceeding, Lymantriidae: a comparison of features of new and old world tussock moths; 1988 June 26-July 1; New Haven, Ct. Gen. Tech. Rep. NE-123. Broomall, PA (now Radnor, PA): U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station: 233-249.

Elkinton, J.S.; Hajek, A.E.; Boettner, G.H.; Simons, E.E. 1991. Distribution and apparent spread of Entomophaga maimaiga (Zygomycetes: Entomophthorales) in gypsy moth (Lepidoptera: Lymantriidae) populations in North America. Environmental Entomology 20(6): 1601-1605.

Elkinton, J.S.; Healy, W.M.; Buonaccorsi, J.P.; Boettner, G.H.; Hazzard, A.M.; Smith, H.R.; Liebhold, A.M. 1996. Interactions among gypsy moths, white-footed mice, and acorns. Ecology 77(8): 2332-2342.

Elkinton, J.S.; Healy, W.M.; Liebhold, A.M.; Buonaccorsi, J.P.; McShea, W.J. (ed.); Healy, W.M. 2002. Gypsy moths and forest dynamics. Johns Hopkins University Press; Baltimore Oak Forest Ecosystems: Ecology and Management for Wildlife: 100-112.

Elliot, R.H.; Iyer, R. 1982. Toxicity of diflubenzuron to nymphs of the migratory grasshopper Melanoplus sanguinipes (Orthoptera: Acrididae). Canadian Entomologist 114(6): 479-484.

Emmett, B.J.; Archer, B.M. 1980. The toxicity of diflubenzuron to honey bee (Apis mellifera L.) colonies in apple orchards. Plant Pathology 29(4): 177-183.

F

Farlow, J. 1976. Ecological impact of Dimilin® on the aquatic fauna of a Louisiana coastal marsh. Doctoral dissertation, Louisiana State University, Agricultural and Mechanical College, Dept. of Entomology. (Unpublished study received Dec 23, 1976; submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL:095650-K). MRID No. 00099678.

Farrar, R.R., Jr.; Martin, P.A.W.; Ridgway, R.L. 1996. Host plant effects on activity of Bacillus thuringiensis against gypsy moth (Lepidoptera: Lymantriidae) larvae. Environmental Entomology 25(5): 1215-1223.

Federal Register (FR), 69 FR 23492-93, April 29, 2004, Notice of Intent to Prepare a Supplement to the Final Environmental Impact Statement for Gypsy Moth Management in the United States: a Cooperative Agreement.

Federal Register (FR), 71 FR 12674-75, March 13, 2006, Revised Notice of Intent to Prepare a Supplement to the Final Environmental Impact Statement for Gypsy Moth Management in the United States: a Cooperative Agreement.

Feicht, D. L.; Fosbroke, S.L.C.; Twery, M.J. 1993. Forest stand conditions after 13 years of gypsy moth infestation. In: Gillespie, A. R.; Parker, G.R.; Pope, Phillips E.; Rink, G., editors. Proceedings, Ninth Central Hardwood Forest Conference; 1993 March 8-10, West Lafayette, IN. General Technical Report NC-161. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 130-144.

Fletcher, D. 1990. 8-Day Dietary LC_{50} with RH-5992 technical in mallard ducklings, Lab Project Number 87RC-0055: 87P-210: BLAL 87 DC 93. Unpublished study prepared by Bio-Life Associates MRID No. 42436237.

Ford-Robertson, F.C., ed. 1971. Terminology of forest science, technology practice and products. Society of American Foresters, Washington, DC.

Fosbroke, D.E.; Hicks, R.R., Jr. 1989. Tree mortality following gypsy moth defoliation in southwestern Pennsylvania. In: Rink, George; Budelsky, Carl A., editors. Proceedings, Seventh Central Hardwood Forest Conference; 1989 March 5-8; Carbondale, IL. General Technical Report. NC-13. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 74-80.

Fuester, R.W.; Taylor, P.B. 1996. Differential mortality in male and female gypsy moth (Lepidoptera: Lymantriidae) pupae by invertebrate natural enemies and other factors. Environmental Entomology 25(2): 536-547.

G

Gaddis, P.K. 1987. Secondary effects of B.t. spray on avian predators: the reproductive success of chickadees. Oregon Department of Agriculture, Plant Division Report, Salem.

Gaddis, P.K.; Corkran, C.C. 1986. Secondary effects of B.t. spray on avian predators: the reproductive success of chestnut-backed chickadees. Oregon Department of Agriculture, Plant Division Report 86-03, Salem.

Gansner, D.A.; Arner, S.L.; Widmann, R.H.; Alerich, C.L. 1993a. After two decades of gypsy moth, is there any oak left? Northern Journal of Applied Forestry 10(4): 184-186.

Gansner, D.A.; Arner, S.L.; Widmann, R.H.; Alerich, C.L. 1993b. What's happening to Pennsylvania's oaks? In: Finley, James C.; Jones, Stephen B., eds. Penn's Woods – Change and Challenge. Proceedings of the Penn State Forest Resources Issues Conference; 1993 April 1-2; State College, PA: 15-22.

Gansner, D.A.; Arner, S.L.; Widmann, R.H.; Alerich, C.L. 1993c. What's up with Billy Penn's oak? In: Twardus, Daniel B., editor. Gypsy Moth News 33: 4-7.

Gansner, D.A.; Herrick, O.W.; White, W.B. 1978. Economic analysis of the gypsy moth problem in the Northeast: IV. Forest stand hazard ratings for gypsy moth. Res. Pap. NE-410. Broomall, PA (Now Radnor, PA): U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station.

Garvey, N. 1992. RH-5992 Technical-subacute (14-day) toxicity to earthworms (Eisenia foetida): Final report: Lab Project Number 91-11: 4003: 90RC-0211. Unpublished study prepared by Springborn Labs, Inc. MRID No. 42991511.

Gaugler, R. 1981. Biological control potential of neoaplectanid nematodes. Journal of Nematology 13(3): 241-249.

Giese, R.L.; Casagrande, R.A. 1981. Egg development and diapause. In: Doane, Charles C.; McManus, Michael L., editors. The gypsy moth: research toward integrated pest management. Technical Bulletin 1548. Washington, D.C.: U.S. Department of Agriculture: 145-146.

Gijswijt, M.J. 1978. Investigations with Dimilin® on bees. Crop Protection Division, Phillips-Duphar B.V., Graveland, the Netherlands.

Gilbert, O. L. 1989. The ecology of urban habitats. New York; Chapman and Hall: 369.

Gill, D.; Bonnett, P. 1973. Nature in the urban landscape. Baltimore, MD: York Press: 36-56.

Glare, T.R.; O'Callaghan, M. 2000. Bacillus thuringiensis: Biology, ecology and safety. John Wiley & Sons, Ltd., New York.

Gordon, R.; Cornect, M. 1986. Toxicity of the insect growth regulator diflubenzuron to the rove beetle Aleochara bilineata, a parasitoid and predator of the cabbage maggot Delia radicum. Entomologia Experimentalis et Applicata 42(2): 179-186.

Gottschalk, K.W. 1988. Gypsy moth and regenerating Appalachian hardwood stands. In: Proceedings. Guidelines for regenerating Appalachian hardwood stands. May 24-26, 1988; Morgantown, WV, West Virginia University: 241-254.

Gottschalk, K.W. 1990a. Economic evaluation of gypsy moth damage in the United States of America. In: proceedings, XIX World Congress; 1990 August 5-11; Montreal, PQ. Canadian IUFRO World Congress Organizing Committee: 235-246.

Gottschalk, K.W. 1990b. Gypsy moth effects on mast production. In: McGee, Charles E., editor. Proceedings of the workshop: Southern Appalachian mast management; 1989 August 14-16; Knoxville, TN. University of Tennessee: 42-50.

Gottschalk, K.W.; MacFarlane, W. R. 1993. Photographic guide to crown condition of oaks: use of gypsy moth silvicultural treatments. General Technical Report NE-168. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station.

Grace, J.R. 1986. The influence of gypsy moth on the composition and nutrient content of litter fall in a Pennsylvania oak forest. Forest Science 32(4): 855-870.

Graves, W.; Smith, G. 1992a. RH-5992 Technical: A 48-hour static acute toxicity test with the Cladoceran (Daphnia magna): Final Report: Lab Project Number 129A-109: 90RC- 0065. Unpublished study prepared by Wildlife International, Ltd. MRID No. 42436241.

Graves, W.; Smith, G. 1992b. RH-5992 Technical: A 96-hour static acute toxicity test with the Bluegill (Lepomis macrochirus): Final Report: Lab Project Number 129A-108: 90RC- 0063. Unpublished study prepared by Wildlife International, Ltd. MRID No. 42436239.

Graves, W.; Smith, G. 1992c. RH-5992 Technical: A 96-hour static acute toxicity test with the Rainbow Trout (Oncorrhynchus mykiss): Lab Project Number 129A-110: 90RC-0064. Unpublished study prepared by Wildlife International, Ltd. MRID No. 42436240.

Griffith, M.B.; Barrows, E.M.; Perry, S.A. 1996. Effects of aerial application of diflubenzuron on emergence and flight of adult aquatic insects. Journal of Economic Entomology 89: 442-446.

Griffith, M.B.; Barrows, E.M.; and Perry, S.A. 2000. Effect of diflubenzuron on flight of adult aquatic insects (Plecoptera, Trichoptera) following emergence during the second year after aerial application. Journal of Economic Entomology 93(6): 1695-1700.

Grushecky, S.T.; Liebhold, A.M.; Greer, R.; Smith, R.L. 1998. Does forest thinning affect predation on gypsy moth (Lepidoptera: Lymantriidae) larvae and pupae? Environmental Entomology 27(2): 268-276.

H

Hagler, L.; Askew, E.W.; Neville, J.R.; Mellick, R.W.; Coppes, R.I.; Lowder, J.F. 1981. Influence of dietary iron-deficiency on hemoglobin, myoglobin, their respective reductases, and skeletal muscle mitochondrial respiration. American Journal of Clinical Nutrition 34(10): 2169-2177.

Hajek, A.E.; Humber, R.A. 1997. Formation and germination of Entomophaga maimaiga azygospores. Canadian Journal of Botany 75(10): 1739-1747.

Hajek, A.E.; Roberts, D.W. 1992. Field diagnosis of gypsy moth (Lepidoptera: Lymantriidae) larval mortality caused by Entomophaga maimaiga and the gypsy moth nuclear polyhedrosis virus. Environmental Entomology 21(4): 706-713.

Hajek, A.E.; Shimazu, M. 1996. Types of spores produced by Entomophaga maimaiga infecting the gypsy moth Lymantria dispar. Canadian Journal of Botany 74(5): 708-715.

Hajek, A.E.; Humber, R.A.; Elkinton, J.S.; May, B.; Walsh, S.R.A.; Silver, J.C. 1990. Allozyme and restriction fragment length polymorphism analyses confirm Entomophaga maimaiga responsible for 1989 epizootics in North American gypsy moth populations. Proceedings of the National Academy of Sciences of the United States of America 87(18): 6979-6982.

Hajek, A.E.; Butler, L.; Wheeler, M.M. 1995a. Laboratory bioassays testing the host range of the gypsy moth fungal pathogen *Entomophaga maimaiga*. Biological Control 5(4): 530-544.

Hajek, A.E.; Renwick, J.A.A.; Roberts, D.W. 1995b. Effects of larval host plant on the gypsy moth (Lepidoptera: Lymantriidae) fungal pathogen, *Entomophaga maimaiga* (Zygomycetes: Entomophthorales). Environmental Entomology 24(5): 1307-1314.

Hajek, A.E.; Bauer, L.; McManus, M.L.; Wheeler, M.M. 1998a. Distribution of resting spores of the *Lymantria dispar* pathogen *Entomophaga maimaiga* in soil and on bark. BioControl 43(2): 189-200.

Hajek, A.E.; Butler, L.; Walsh, S.R.A.; Silver, J.C.; Hain, F.P.; Hastings, F.L.; Odell, T.M.; Smitley, D.R. 1998b. Host range of the gypsy moth (Lepidoptera: Lymantriidae) pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) in the field versus laboratory. Environmental Entomology 25(4): 709-721.

Hajek, A.E.; Olsen, C.H.; Elkinton, J.S. 1999. Dynamics of airborne conidia of the gypsy moth (Lepidoptera: Lymantriidae) fungal pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthorales). Biological Control 16(1): 111-117.

Hajek, A.E.; Wheeler, M.M.; Eastburn, C.C.; Bauer, L.S. 2001. Storage of resting spores of the gypsy moth fungal pathogen *Entomophaga maimaiga*. Biocontrol Science Technology; October 11.

Hall, D. 1986. Toxicity of Two Insect Growth Regulators on *Ceriodaphnia dubia*. Unpublished study prepared by Colorado State University, Department of Fishery & Wildlife. MRID No. 40130601.

Hansen, S.R.; Garton, R.R. 1982. Ability of standard toxicity tests to predict the effects of the insecticide diflubenzuron on laboratory stream communities. Canadian Journal of Fisheries and Aquatic Sciences 39: 1273-1288.

Hastings, F.L.; Hain, F.P; Smith, H.R.; Cook, S.P.; Monahan J.F. 2002. Predation of gypsy moth (Lepidoptera: Lymantriidae) pupae in three ecosystems along the southern edge of infestation. Environmental Entomology 31(4): 668-675.

Heichel, G. H.; Turner, Neil C. 1976. Phenology and leaf growth of defoliated hardwood trees. In: Anderson, John F.; Kaya, Harry K., editors. Perspectives in forest entomology. New York; Academic Press: 31-40.

Hendersen, C.; Smith, H.D.; Jorgensen, C.D. 1977. Small mammal responses to experimental pesticide applications in coniferous forests. Final report Douglas-fir tussock moth R&D Program RA-8, Pacific Southwest Forest and Range Experiment Station, Berkeley.

Herms, C.P.; McCullough, D.G.; Bauer, L.S.; Haack, R.A.; Miller, D.L.; Dubois, N.R. 1997. Susceptibility of the endangered Karner blue butterfly (Lepidoptera: Lycaenidae) to *Bacillus thuringiensis* var.*kurstaki* used for gypsy moth suppression in Michigan. Great Lakes Entomologist 30(4): 125-141.

Herms, D.A. 2003. Assessing management options for gypsy moth. Pesticide Outlook, February: 14-18.

Hernandez, E.; Ramisse, F.; Gros, P.; Cavallo, J.D. 2000. Super-infection by *Bacillus thuringiensis* H34 or 3a3b can lead to death in mice infected with influenza A virus. FEMS Immunology Med Microbioloby 29: 177-161.

Herrick, O.W. 1982. Hazard rating forest trees threatened with gypsy moth invasion. In: Coping with the gypsy moth. Proceedings of the Penn State Forest Resources Issues Conference; 1982 February 17-18; University Park, PA: 38-42.

Herrick, O.W.; Ganser, D.A. 1986. Gypsy moth on a new frontier: forest tree defoliation and mortality, Northern Journal of Applied Forestry 4: 128-133.

Herrick, O.W.; Gansner, D.A. 1987. Morality risks for forest trees threatened with gypsy moth infection. Research Note NE-388. Broomall, PA (now Radnor, PA): U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station.

Herrick, O.W.; Gansner, D.A. 1988. Changes in forest condition associated with gypsy moth on new frontiers of infestation. Northern Journal of Applied Forestry 5(1): 59-61.

Hix, D.M.; Fosbroke, D.E.; Hicks, R.R., Jr.; Gottschalk, K.W. 1991. Development of regeneration following gypsy moth defoliation on Appalachian Plateau and ridge valley hardwood stands. In: McCormick, Larry H.; Gottschalk, Kurt W., editors. Proceedings, 8th Central Hardwood Forest Conference; 1991 March 3-6; University Park, PA. General Technical Report. NE-148. Radnor, PA: U.S. Department of Agriculture, Forest Service. Northeastern Forest Experiment Station: 347-359.

Hofacker, T.H. 1994. Entomologist, U.S. Department of Agriculture, Forest Service, Forest Pest Management Staff, Washington, DC. [Statement made at meeting with EIS Team]. 14 June.

Holmes, S.B. 1998. Reproduction and nest behavior of Tennessee warblers *Vermivora peregrina* in forests treated with Lepidoptera-specific insecticides. J. Applied Ecology 35: 185-194.

Houston, D.R. 1981a. Oak decline and mortality. In: Doane, Charles C.; McManus, Michael L., editors. The gypsy moth: research toward integrated pest management. Tech. Bul. 1584. Washington, DC: U.S. Department of Agriculture: 217-219.

Houston, D.R. 1981b. Forest stand relationships. In: Doane, Charles C.; McManus, Michael L., editors. The gypsy moth; research toward integrated pest management. Tech. Bul. 1584. Washington, DC: U.S. Department of Agriculture: 267-293.

Hurd, M.K.; Perry, S.A.; Perry, W.B. 1996. Non-target effects of a test application of diflubenzuron to the forest canopy on stream macroinvertebrates. Environonmental Toxicology and Chemistry 15(8): 1344-1351.

Hutchens, J.J., Jr.; Benfield, E.F. 2000. Effects of forest defoliation by the gypsy moth on detritus processing in southern Appalachian streams. American Midland Naturalist 143(2): 397-404.

I

Ivie, G. Wayne: Bull, Done L.; Veech, Joseph A. 1980. Fate of diflubenzuron in water. Journal of Agricultural and Food Chemistry 28 (2): 330-337.

J

James, R.R.; Miller, J.C.; Lighthart, B. 1993. *Bacillus thuringiensis* var. *kurstaki* affects a beneficial insect, the cinnabar moth (Lepidoptera: Arctiidae). Journal of Economic Entomology 86(2): 334-339.

Jepson, P.C.; Yemane, A. 1991. Toxicity of diflubenzuron to nymphs of the desert locust *Schistocerca gregaria* Forsk (Orthoptera: Acrididae). Pesticide Science 34(1):92-93.

Johnson, W.; Finley, M.R. 1980. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. USDI Publication 137, Washington DC. Cited in U.S. EPA 1997.

K

Kaya, H.K.; Readon, R.C. 1982. Evaluaton of *Neoaplectana carpocapsae* for biological control of the western spruce budworm, (*Choristoneura occidentalis*): ineffectiveness and persistence of tank mixes. Journal of Nematology 14(4): 95-597.

Kay, H.K.; Hara, A.H.; Readon, R.C. 1981. Laboratory and field evaluations on *Neoaplectana carpocapsae* (Rhabditda: Steinernematidae) against the elm leaf beetle (Coleoptera: Chrysomelidae) and the western spruce budworm (Lepidoptera: Tortricidae). The Canadian Entomologist 113(3): 787-793.

Keever, D.W.; Bradley, J., Jr.; Ganyard, M.C. 1977. Effects of diflubenzuron (Dimilin®) on selected beneficial arthropods in cotton fields. Environmental Entomology 6(5): 732-736.

Keller, L. 1998. Literature Summary for Tebufenozide (RH-5992). Rohm and Haas Report No. 98R-1055. Report submitted by John W. Long, Product Development Manager, Rohm and Hass: to David F. Thomas, USDA Forest Service, Dec. 1, 1999.

Khakoo, G.A.; Maconochie, I.K.: Jaffer, P. 1993. An unusual blue baby. Journal of Royal Society of Medicine (London) 86(12): 730-731.

Knipling, E. F. 1979. The basic principles of insect population suppression and management. Agricultural Handbook 512. Washington, DC: U.S. Department of Agriculture.

Kolodny-Hirsch, D.M.; Webb, R.E.; Olsen, R.; Venables, L. 1990. Mating disruption of gypsy moth (Lepidoptera: Lymantriidae) following repeated ground application of racemic disparlure. Journal of Economic Entomology 83(5): 1972-1976.

Kozlowski, T.T. 1969. Tree physiology and forest pests. Journal of Forestry 67(2): 118-123.

Kreutzweiser, D.P.; Thomas, D.R. 1995. Effects of a new molt-inducing insecticide, tebufenozide, on zooplankton communities in lake enclosures. Ecotoxicology 4: 307-328.

Kreutzweiser, D.P.; Holmes, S.B.; Capell, S.S.; Eichenberg, D.C. 1992. Lethal and sublethal effects of *Bacillus thuringiensis* var. *kurstaki* on aquatic insects in laboratory bioassays and outdoor stream channels. Bulletin of Environmental Contamination and Toxicology 49: 252-258.

Kreutzweiser, D.P.; Capell, S.S.; Thomas, D.R.; Wainio-Keizer, K.L. 1993. Effects of *B.t.k.*. on aquatic microbial activity, detrital decomposition, and invertebrate communities. NAPIAP Proj. NA-25. Sault Ste., Marie, Ontario: Forestry Canada, Forest Pest Management Institute.

Kreutzweiser, D.P.; Capell, S.S.; Wainio-Keizer, K.L.; Eichenberg, D.C. 1994. Toxicity of a new molt-inducing insecticide (RH-5992) to aquatic macroinvertebrates. Ecotoxicology and Environmental Safety 28: 14-24.

Kreutzweiser, D.P.; Ebling, P.M.; Holmes, S.B. 1997. Infectivity and Effects of Gypsy Moth and Spruce Budworm Nuclear Polyhedrosis Viruses Ingested By Rainbow Trout. Ecotoxicology. 38 (1): 63-70.

Kuijpers, L. 1988. The acute toxicity of diflubenzuron to Daphnia magna: Laboratory Project ID: C.303.51.008. Unpublished study prepared by Duphar B.V. MRID No. 40840502.

Kuijpers, L. 1989. The side-effects of diflubenzuron (Dimilin®) on bees: A review. Philips-Duphar B.V., The Netherlands, Crop Protection Division, Report No. 56635/07/89.

Kulman, H.M. 1971. Effects of insect defoliaton on growth and mortality of trees. Annual Review of Entomology 16: 289-324.

Kumar, S.; Dahiya, B.; Chauhan, R.; Jaglan, M.S. 1994. Ovicidal action of diflubenzuron against *Helicoverpa armigera* (Lepidoptera: Noctuidae). Agriculural Science Digest.

L

LaChance, L.E. 1985. Genetic methods for control of Lepidopteran species: status and potential. ARS-28. U.S. Department of Agriculture, Agricultural Research Service. Available from: NTIS, Springfield, VA.

Lahr, J.; Diallo, A.O.; Gadji, B.; Diouf, P.S.; Bedaux, J.J.M.; Badji, A.; Ndour, K.B.; Andreasen, J.E.; Straalen, Nmvan. 2000. Ecological effects of experimental insecticide applications on invertebrates in Sahelian temporary ponds. Environmental Toxicology and Chemistry. 19: 1278-1289.

Lautenschlager, R.A.; Rothenbacher, H.; Podgwaite, J.D. 1976. The response of birds to aerially applied nuclear polyhedrosis virus of the Gypsy Moth, *Lymantria dispar* L. (U.S. Forest Service, Northeastern Forest Experiment Station, Forest Insect and Disease Laboratory and Pennsylvania State Univ., Dept. of Veterinary Pathology for USFS. Unpublished study; CDL:227336-AN). MRID No. 00066108.

Lautenschlager, R.A.; Kircher, C.H.; Podgwaite, J.D. 1977. Effect of nucleopolyhedrosis virus on selected mammalian predators of the gypsy moth. Res. Pap. NE-377. Upper Darby, PA (now Radnor, PA): U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station.

Leonard, D.E. 1981. Bio-ecology of the gypsy moth. In: Doane, Charles C.; McManus, Michael L., eds. The gypsy moth: research toward integrated pest management. Technical Bulletin 1584. Washington, DC: U.S. Department of Agriculture: 9-29.

Liebhold, A.M.; Gottschalk, K.W.; Mason, D.A.; Bush, R.R. 1997. Forest susceptibility to the gypsy moth. Journal of Forestry 95(5): 20-24.

Liebhold, A.M.; Gottschalk, K.W.; Muzika, R.; Montgomery, M.E.; Young, R.; O'Day, K.; Kelley, B. 1995. Suitability of North American tree species to the gypsy moth: a summary of field and laboratory tests. Gen. Tech. Rep. NE-211. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station.

Lincoln, R. J.; Boxshall, G.A. 1987. The Cambridge illustrated dictionary of natural history. Cambridge University Press.

Little, E.L., Jr. 1979. Checklist of United Sates trees (native and naturalized). Agricultural Handbook 541. Washington, DC: U.S. Department of Agriculture.

Livingston, R.; Koenig, C. 1977. Life cycle toxicity tests concerning the acute and chronic effects of a pesticide (TH-6040) on the mummichug (*Fundulus heteroclitus* Walbaum), and egg-laying topminnow. (Unpublished study received Feb 6, 1978 under 1481259; prepared by Environmental Planning & Analysis, submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL:096787-M). MRID No. 00099722.

Lo, S.C.; Agar, N.C. 1986. NADH-methemoglobin reductase activity in the erythrocytes of newborn and adult mammals. Experientia 42(11-12): 1264-1265.

Lovett, G.M.; Christiansen, L.M.; Groffman, P.M.; Jones, G.G.; Hart, J. E.; Mitchell, M.J. 2002. Insect defoliation and nitrogen recycling in Forests. Bioscience 52: 335-341.

M

Maddox, J.V.; Baker, M.D.; Jeffords, M.R.; Kuras, M.; Linde, A.; Solter, L.F.; McManus, M.L.; Vavra, J.; Vossbrinck, C.R. 1999. Nosema portugal, NSP, isolated from gypsy moths (*Lymantria dispar* L.) collected in Portugal. Journal of Invertebrate Pathology. 73.

Majori, G.; Romi, R.; Ali, A. 1984. Toxicity of the IGR diflubenzuron to neonate, adult, and gravid female Daphnia magna Straus (Cladocera: Daphniidae) in the laboratory. Proceedings and Papers on the 52nd Annual Conference of the California Mosquito and Vector Control Association: 68-70.

Martinat, P.J.; Coffman, C.C.; Dodge, K.; Cooper, R.J.; Whitmore, R.C. 1988. Effect of diflubenzuron on the canopy arthropod community in a central Appalachian forest. Journal of Economic Entomology 81(2); 261-267.

Mastro, V.C., Schwalbe, C.P.; ODell, T.M. 1981. Sterile-male technique. In: Doane, Charles C.; McManus, Michael L., eds. The gypsy moth: research toward integrated pest management. Tech. Bull. 1584. Washington, DC: U.S. Department of Agriculture; 669-679.

Matthenius, J.C., Jr. 1975. Effects of Dimilin® on honey bees. (New Jersey, Dept. of Agriculture, unpublished study; CDL: 094969-K). MRID No. 00038216.

Mazzone, H.M.; Tignor, G.H.; Shope, R.E.; Ran, I.C.; Hess, W.R. 1976. A serological comparison of the nuclear polyhedrosis viruses of the gypsy moth and the European pine sawfly with arthropod-borne and other viruses. Environmental Entomology 5(2): 281-282.

McAlonan, W. 1975. Effects of two insect growth regulators on some selected saltmarsh non-target organisms. (Unpublished study received Jul 31, 1978 under 148-1259; submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL:234511-AA). MRID No. 00099895.

McFadden, M.W.; McManus, M.E. 1991. An insect out of control? The potential for spread and establishment of the gypsy moth in new forest areas in the United States. In: Baranchikov, Yuri N.; Mattson, William J.; Hain, Fred P.; Payne, Thomas L., editors. Forest insect guilds: patterns of interaction with host trees. General Technical Report NE-153. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station: 172-186.

McManus, M.L.; Leellen, S. 2003. Microsporidian pathogens in European gypsy moth populations. In: Proceedings, Ecology, Survey and Management of Forest Insects (Michael L. McManus and Andrew Liebhold, editors). U.S. Department of Agriculture GTR NE-311.

McManus, M.L.; McIntyre, T. 1981. Introduction. In: Doane, Charles C.; McManus, Michael L., eds. The gypsy moth: research toward integrated pest management. Technical Bulletin 1584. Washington, DC: U.S. Department of Agriculture; 1-7.

McManus, M.; Schneeberger, N.; Reardon, R.; Mason, G. 1989. Gypsy moth. Forest Insect Disease Leaflet 162. Washington, DC: U.S. Department of Agriculture, Forest Service.

McShae, W.J. 2000. The influence of acorn production on annual variation in rodent and bird populations. Ecology 81: 228-238.

McShea, W.; Rappole, John H. 1992. White-tailed deer as keystone species within forest habitats of Virginia. Virginia Journal of Science 43(1B): 177-186.

McShea, W.J.; Schwede, G. 1993. Variable acorn corps: responses of white-tailed deer and other mast consumers. Journal of Mammalogy 74(4): 999-1006.

Miller, J.C. 1990. Field assessment of the effects of a microbial pest control agent on non-target Lepidoptera. American Entomologist 36 (summer): 135-139.

Moffett, M. 1995. Effects, persistence and distribution of diflubenzuron in littoral enclosures: Final Report. Unpublished study prepared by U.S. EPA and University of Wisconsin-Superior. MRID No. 44386201.

Moore, R.B. 1977. Determination of the effects of nuclear polyhedrosis virus in Trout and Bluegill Sunfish under laboratory conditions. Cooperative Agreement No. 42-213.(Unpublished study received Aug 18, 1977 under 27582-2; prepared by Essex Marine Laboratory, Inc. and U.S. Forest Service, Northeastern Area, State and Private Forestry; submitted by Parker Livestock Supply, Inc. MRID No. 00054565.

Montgomery, M.E. 1991. Variations in the suitability of tree species for the gypsy moth. In: Gottschalk, Kurt W., Twery, Mark J.; Smith, Shirley I., editors. Proceedings, U.S. Department of Agriculture Interagency Gypsy Moth Research Review; 1990 January 22-25; East Windsor, CT. Gen. Tech. Rep. NE-146. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station.

Murray, K.F.; Christie, D.L. 1993. Dietary protein intolerance in infants with transient methemoglobinemia and diarrhea. Journal of Pediatrics 122(1): 90-92.

Muzika, R.M.; Liebhold, A.M.1999. Changes in radial increment of host and nonhost tree species with gypsy moth defoliation. Canadian Journal of Forest Research 29(9): 1365-1373.

N

Nagy, L.R.; Smith, K.G. 1997. Effects of insecticide-induction reduction in Lepidopteran larvae on reproductive success of hooded warblers. Auk.114(4): 619-627.

Naidoo, R.; Lechowicz, M.J. 2001. Effects of gypsy moth on radial growth of deciduous trees. Forest Science Aug 47(3): 338-348.

Nation, J.L.; Robinson, F.A.; Yu, S.J.; Bolten, A.B. 1986. Influence upon honeybees of chronic exposure to very low levels of selected insecticides in their diet. Journal of Apiculture Research. 25(3): 170-177.

Nichols, J.O. 1961. The gypsy moth in Pennsylvania: its history and eradication. Misc. Bull. 4404. Harrisburg, PA: Pennsylvania Department of Agriculture.

Nichols, J.O. 1980. The gypsy moth. Harrisburg, PA: Commonwealth of Pennsylvania, Department of Environmental Resources, Bureau of Forestry.

Nichols, S.W., compiler. 1989. The Torre-Bueno Glossary of Entomology, including Supplement A by George S. Tulloch. New York Entomological Society, with the American Museum of Natural History.

Nilsson, A.; Engberg, G.; Henneberg, S.; Danielson, K.; De Ferdier, C. H. 1990. Inverse relationship between age-dependent erythrocyte activity of methaemoglobin reductase and prilocaine-induced methemoglobinaemia during infancy. British Journal of Anaesthesia (London) 64(1): 72-76.

North, D.T. 1975. Inherited sterility in Lepidoptera. Annual Review of Entomology 20: 167-182.

Norton, M.L.; Bendell, J.F.; Bendell-Young, L.I.; Leblanc, C.W. 2001. Secondary effects of the pesticide *Bacillus thuringiensis* kurstaki on chicks of spruce grouse (*Dendragapus canadensis*). Archives of Environmental Contamination and Toxicology 41(3): 369-373.

O

Oberlander, H.; Silhacek, D.L.; Leach, C.E. 1998. Interactions of ecdysteroid and juvenoid agonists in *Plodia interpunctella* (Hubner). Archives of Insect Biochemistry and Physiology. 38(2): 91-99.

O'Connor, T.F.; Moore, R.B. 1975. The effects of Dimilin® on small mammals. In: The environmental impact of Dimilin® (TH 6040) on a forest and aquatic ecosystem. Lake Ontario Environmental Laboratory. LOTEL Report Number. 210: 120-139.

Ostfeld, R.S.; Jones, C.G.; Wolff, J.O. 1996. Of mice and mast: ecological connections in eastern deciduous forests. BioScience 46(5): 323-330.

Otvos, I.S.; Vanderveen, S. 1993. Environmental report and current status of *Bacillus thuringiensis* var. *kurstaki* use in control of forest and agricultural insect pests. Victoria, BC: Forestry Canada and Province of British Columbia, Ministry of Forests.

P

Paananen, D.M.; Fowler, R.F., Wilson, L.F. 1987. The aerial war against eastern region forest insects, 1921-1986. Journal of Forest History 31(4): 173-186.

Parker, J. 1981. Effects of defoliation on oak chemistry. In: Doane, Charles C.; McManus, Michael L., editors. The gypsy moth: research toward integrated pest management. Technical Bulletin 1584. Washington, DC. U.S. Department of Agriculture: 219-225.

Payne, N.; Retnakaran, A.; Cadogan, B. 1997. Development and evaluation of a method for the design of spray applications: aerial tebufenozide applications to control the Eastern Spruce Budworm, *Choristoneura fumiferana* (Clem.). Crop Protection 16 (3): 285-290.

Podgwaite, J., Microbiologist, USDA Forest Service, [Conversation with Joseph L. Cook]. 28 July 2004.

Podgwaite, J.D.; Campbell, R.W. 1972. The disease complex of the gypsy moth: II. Aerobic bacterial pathogens. Journal of Invertebrate Pathology 20: 303-308.

Podgwaite, J.; Galipeau, P. 1978. Effect of nucleopolyhedrosis virus on two avian predators of the gypsy moth (Forest Service Research Note NE-251; also in unpublished submission received Sep 11, 1979 under 27586-2; submitted by U.S. Forest Service, Washington, DC; CDL:240994-I). MRID No. 00134318.

Powell, D.S.; Faulkner, J.L.; Darr, D.R.; Zhu, Z.; MacCleery, D.W. 1993. Forest resources of the United States, 1992. Gen. Tech. Rep. RM-234. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station.

Q

Quimby, J.W. 1985. Tree mortality in Pennsylvania forests defoliated by gypsy moth—a 1984 update. In: Forest management decisions based on future forest product requirements. Proceedings of the Penn Sate Forest Resources Issues Conference; 1985 March 19-20; State College, PA: 95-105.

Quimby, J.W. 1987. Impact of gypsy moth defoliation on forest stands. In: Fosbroke, Sandra; Hicks, Ray , Jr., eds. Proceedings on the workshop: Coping with the gypsy moth in the new frontier; 1987 August 4-6; Morgantown, WV. West Virginia University Books: 21-29.

Quimby, J.W. 1993. Tree morality following gypsy moth epidemic. In: Twardus, Daniel B., ed. Gypsy Moth News 33: 8-12.

R

Rastall, K.; Kondo, V.; Strazanac, J.S.; Butler, L. 2003. Lethal effects of biological insecticide applications on non-target lepidopterans in two Appalachian forests. Environmental Entomology 32(6): 1364-1369.

Reardon, R.C. 1981. 6.1 Parasites: introduction. In: Doane, Charles C.; McManus, Michael L., eds. The gypsy moth: research toward integrated pest management. Technical Bulletin 1584. Washington, DC: U.S. Department of Agriculture: 300-301.

Reardon, R.C. 1991. Appalachian gypsy-moth integrated pest-management project. Forest Ecology and Management 39: 107-112.

Reardon, R.C., ed. 1995. Effects of diflubenzuron on non-target organisms in broadleaf forested watersheds in the Northeast. USDA National Center of Forest Health Management; November 1995. FHM-CN-05-95.

Reardon, R.C. 1998. Using mating disruption to manage gypsy moth : a review. FHTET (Series); 98-01. Morgantown, WV: Forest Health Technology Enterprise Team, USDA, Forest Service.

Reardon, R.; Hajek, A. 1993. *Entomophaga maimaiga* in North America: a review. NA-TP-15-93. Radnor, Pa: U.S. Department of Agriculture, Forest Service, Northeastern Area.

Reardon, R.; Hajek, A.E. 1995. *Entomophaga maimaiga* in North America: a review. In: Twardus, Daniel B., editor Gypsy Moth News 39: 3-4.

Reardon, R. C.; Mastro, V.C. 1993. Development and status of the sterile insect technique for managing gypsy moth. NA-TP-13-93 [Radnor, PA]: U.S. Department of Agriculture, Forest Service, Northeastern Area State and Private Forestry.

Reardon, R.; Podgwaite, J. 1992. The gypsy moth nucleopolyhedrosis virus product. NA-TP-02-92. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Area.

Reardon, R.C.; Kaya, H.K.; Fusco, R.A.; Lewis, F.B. 1986. Evaluation of *Steinernema feltiae* and *S. bibious* (Rhabditida: Steinernematidae) for suppression of *Lymantria dispar* (Lepidoptera: Lymantriidae) in Pennsylvania, U.S.A. Agriculture, Ecosystems and Environment 15: 1-9.

Reardon, R; Venables, L.; Roberts, A. 1993. The Maryland Integrated Pest Management Gypsy Moth Project: 1983-1987. NA-TP-07-93. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Area State and Private Forestry.

Reardon, R.; Dubois, N.; McLane, W. 1994. *Bacillus thuringiensis* for managing gypsy moth: a review. FHM-NC-01-94. Morgantown, WV: U.S. Department of Agriculture, Forest Service, National Center of Forest Health Management.

Reardon, R.C.; Podgwaite, J.; Zerillo R. 1996. Gypchek – the gypsy moth polyhedrosis virus product. FHTET-96-16.

Reardon, R.; Cowan, D.; McLane, W.; Talley, S. 2000. Efficacy and deposit assessment of tebufenozide against gypsy goth (Lepidoptera: Lymantriidae). FHTET-97-06.

Redfern, R.E.; Demilo, A.B.; Borkovec, A.B. 1980. Large milkweed bug: effects of diflubenzuron and its analogues on reproduction. Journal of Economic Entomology 73: 682-683.

Redman, A.M.; Scriber, J.M. 2000. Competition between the gypsy moth, *Lymantria dispar*, and the northern tiger swallowtail, Papilio canadensis: interactions mediated by host plant chemistry, pathogens, and parasitoids. Oecologia 125(2): 218-228.

Rehder, A. 1951. Manual of cultivated trees and shrubs hardy in North America. 2nd edition. New York: Macmillan Co.

Reiner, H.K.; Parke, G.S.E. 1975. Report: Static 96-hour toxicity study of Thompson- Hayward Chemical Company. sample TH 6040 in Fathead Minnows: Laboratory No. 5E-6095. (Unpublished study received Jul 31, 1978 under 148-1259; prepared by Cannon Laboratories, Inc., submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL:234513-S); MRID No. 00060376. Cited in U.S. EPA 1997a.

Reinert, K. 1995a. (RH-5992 Technical) Reproduction study in bobwhite quail: Lab Project Number 94RC-0023: RH59BWR-394: HWA 417-492. Unpublished study prepared by Ecological Planning and Toxicology, Inc.; MRID No. 43781701.

Reinert, K. 1995b. (RH-5992 Technical) Avian reproduction toxicity: Supplement to Rohm and Haas Report No. 94RC-0023: Lab Project Number 94RC-0023B. Unpublished study prepared by Rohm and Haas Co.; MRID No. 43781702.

Retnakaran, A.; Macdonald, A.; Tomkins, W.L.; Davis, C.N.; Brownwright, A.J.; Palli, S.R. 1997a. Ultrastructural effects of a non-steroidal ecdysone agonist, RH-5992, on the sixth instar of the Spruce budworm, *Choristoneura fumiferana*. Journal of Insect Physiology 43(1): 55-68.

Retnakaran, A.; Smith, L.F.R.; Tomkins, W.L.; Primavera, M.J.; Palli, S.R.; Payne, N.; Jobin, L. 1997b. Effect of RH-5992, a nonsteroidal ecdysone agonist, on the Spruce budworm, *Toneura fumiferana* (Lepidoptera: Tortricidae): Laboratory, greenhouse and ground spray. Canadian Entomologist. 129(5): 871-885.

Rodenhouse, N.L.; Holmes, R.T. 1992. Results of experimental and natural food reductions for breeding black-throated blue warblers. Ecology 73(2): 357-372.

Rumpf, S.; Frampton, C.; Dietrich, D.R. 1998. Effects of conventional insecticides and insect growth regulators on fecundity and other life-table parameters of *Micromus tasmaniae* (Neuroptera: Hemerobiidae). Journal of Economic Entomology 91: 34-40.

S

Sample, B.E.; Butler, L.; Whitmore, R.C. 1993a. Effects of an operational application of Dimilin® on non-target insects. The Canadian Entomologist 125(2): 173-179.

Sample, B.E.; Butler, L.; Zivkovich, C.; Whitmore, R.C. 1993b. Evaluation of *Bacillus thuringiensis* and defoliation effects on native Lepidoptera. NA-TP-10-93. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Area State and Private Forestry.

Sample, B.E.; Whitmore, R.C. 1993. Food habits of the endangered Virginia big-eared bat in West Virginia, Journal of Mammalogy 74(2): 428-435.

Sample, B.E.; Butler, L.; Zivkovich, C.; Whitmore, R.C.; Reardon, R. 1996. Effects of *Bacillus thuringiensis* (Beliner) var. *kurstaki* and defoliation by the gypsy moth (*Lymantria dispar* (L.), Lepidoptera: Lymantridae) on native arthropods in West Virginia. The Canadian Entomologist 128: 573-592.

Schroeder, W. 1978. Letter sent to J. Taylor dated Feb 13, 1978: Dimilin® sprays applied to citrus, effect on bees. (U.S. Agricultural Research Service, Southern Region, Horticultural Research Laboratory; unpublished study; CDL:096960-A). MRID No. 00099731.

Schroeder, W.J.; Sutton, R.A.; Beavers, J.B. 1980. *Diaprepes abbreviatus*: fate of diflubenzuron and effect on nontarget pests and beneficial species after application to citrus for weevil control. Journal of Economic Entomology 73(5): 637-638.

Schweitzer, D.F. 1988. Element stewardship abstract for *Lymantria dispar* gypsy moth. Arlington, VA: The Nature Conservancy.

Seidel, G.E.; Whitmore, R.C. 1995. Effects of Dimilin® application on white-footed mouse populations in a central Appalachian forest. Environmental Toxicology and Chemistry 14(5): 793-799.

Sharov, A.A.; Liebhold, A.M. 1998. Model of slowing the spread of gypsy moth (Lepidoptera: Lymantriidae) with a barrier zone. Ecological Applications 8(4): 1170-1179.

Sharov, A.A.; Leonard, D.; Liebhold, A.M.; Clemens, N.S. 2002a. Evaluation of preventive treatments in low-density gypsy moth populations using pheromone traps. Journal of Economic Entomology 95(6): 1205-1215.

Sharov, A.A.; Leonard, D.; Liebhold, A.M.; Roberts, E.A.; Dickerson, W. 2002b. "Slow the Spread:" a national program to contain the gypsy moth. Journal of Forestry 2002, 100(5): 30-35.

Sharov, A.A.; Thorpe, K.W.; Tcheslavskaia, K. 2002c. Effect of synthetic pheromone on gypsy moth (Lepidoptera: Lymantriidae) trap catch and mating success beyond treated areas. Environmental Entomology 31(6): 1119-1127.

Sharpe, W.E. 1982. Effects of gypsy moth defoliation in Pennsylvania on water quality and quantity, In Proceedings of Coping With Gypsy Moth in the New Frontier; 1982 Feb 17-18; The Pennsylvania State University; 95-102.

Sheath, R.G.; Burkholder, J.A.M.: Morison, M.O.; Steinman, A.D.; VanAlstyne, K.L. 1986. Effect of tree canopy removed by gypsy moth larvae on the macroalgae of a Rhode Island headwater stream, Journal of Phycology 22: 567-570.

Shimazu, M.; Soper, R.S. 1986. Pathogenicity and sporulation of *Entomophaga maimaiga* Humber, Shimazu, Soper and Hajek (Entomophthorales: Entomophthoraceae) on larvae of the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae). Applied Entomology and Zoology 21(4): 589-596.

Showalter, C.R.; Whitmore, R.C. 2002. The effect of gypsy moth defoliation on cavity-nesting bird communities. Forest Science 48(2): 273-281.

Sinha, S.N.; Lakhani, K.H.; Davis, N.K. 1990. Studies on the toxicity of insecticidal drift to the first instar larvae of the Large White butterfly *Pieris brassicae* (Lepidoptera: Pieridae). Annals of Applied Biology 116:27-41.

Smagghe, G.; Degheele, D. 1994a. Action of a novel non-steroidal ecdysteroid mimic, tebufenozide (RH-5992), on insects of different orders. Pesticide Science 42(2): 85-92.

Smagghe, G.; Degheele, D. 1994b. The significance of pharmacokinetics and metabolism to the biological activity of RH-5992 (tebufenozide) in *Spodoptera exempta, Spodoptera exigua* and *Leptinotarsa ineata.* Pesticide Biochemistry and Physiology 49(3): 224-234.

Smagghe, G.; Eelen, H.; Verschelde, E.; Richter, K.; Degheele, D. 1996. Differential effects of nonsteroidal ecdysteroid agonists in Coleoptera and Lepidoptera: Analysis of evagination and receptor binding in imaginal discs. Insecticide Biochemistry and Molecular Biology 26(7): 687-695.

Smagghe, G.; Vinuela, E.; Budia, F.; Degheele, D. 1997. Effects of the non-steroidal ecdysteroid mimic tebufenozide on the Tomato looper *Deixis chalcites* (Lepidoptera: Noctuidae): An ultrastructural analysis. Archives of Insect Biochemistry and Physiology. 35: 179-190.

Smith, D.M. 1986. The practice of silviculture. 8th edition. New York: John Wiley and Sons: 1, 47-49, 87-88.

Smith, H.R. 1985. Wildlife and the gypsy moth. Wildlife Society Bulletin 13(3): 166-174.

Smith, R.P. 1996. Toxic responses of the blood. In: Casarett and Doull's Toxicology: The Basic Science of Poisons. 5th Edition. McGraw-Hill, Health Professions Division, New York: 335-353.

Smith, H.R.; Lautenschlager, R.A. 1978. Predators of the gypsy moth. Agric. Handbook 534. Washington, DC: U.S. Department of Agriculture.

Smith, H.R.; Lautenschlager, R.A. 1981. Gypsy moth predators. In: Doane, Charles C.; McManus, Michael L., editors. The gypsy moth: research toward integrated pest management. Technical Bulletin 1584. Washington, DC: U.S. Department of Agriculture: 96-124.

Smitley, D.R.; Bauer, L.S.; Hajek, A.E.; Sapio, F.J.; Humber, R.A. 1995. Introduction and establishment of *Entomophaga maimaiga*, a fungal pathogen of gypsy moth (Lepidoptera: Lymantriidae) in Michigan Environmental Entomology 24(6): 1685-1695.

Snow, J.W.; Jones, R.L.; North, D.T.; Holt, G.G. 1971. Effects of irradiation on ability of adult male corn earworms to transfer sperm and field attractiveness of females mated to irradiated males. Journal of Economic Entomology 65(3): 906-908.

Song, M.Y.; Star, J.D.; Brown, J.J. 1997. Comparative toxicity of four insecticides, including imidacloprid and tebufenozide, to aquatic arthropods. Environmental Toxicology and Chemistry. 16(12): 2494-2500.

Soper, R.S.; Shimazu, M.; Humber, R.A.; Ramos, Mark E.; Hajek, A.E. 1988. Isolation and characterization of *Entomophaga maimaiga* sp. Nov., a fungal pathogen of gypsy moth, *Lymantria dispar*, from Japan. Journal of Invertebrate Pathology 51(3): 229-241.

Sopuck, L.; Ovaska, K.; Whittington, B. 2002. Responses of songbirds to aerial spraying of the microbial insecticide *Bacillus thuringiensis* var. *kurstaki* (foray 48B(R)) on Vancouver Island, British Columbia, Canada. Environmental Toxicology and Chemistry 21(8): 1664-1672.

Staley, J.M. 1965. Decline and mortality of red and scarlet oaks. Forest Service 11(2): 2-17.

Stephens, G.R.; Hill, D.E. 1971. Drainage, drought, defoliation, and death in unmanaged Connecticut forests. Bulletin 718. New Haven: Connecticut Agricultural Experiment Station.

Stevenson, J.H. 1978. The acute toxicity of formulated pesticides to worker honey bees. (*Apis mellifera)*. Plant Pathology, 27: 38-40. Summarized in WHO 1997.

Strazanac, John S.; Butler, Linda. 2005. Long-term evaluation of the effects of *Bacillus thuringiensis kurstaki*, gypsy moth nucleopolyhedrosis virus product Gypchek, and *Entomophaga maimaiga* on nontarget organisms in mixed broadleaf-pine forests in the central Appalachians. FHTET-2004-14. [Morgantown, WV]: USDA Forest Service, Forest Health Technology Enterprise Team; 81 p.

Streams, F.A. 1976. Susceptibility of aquatic invertebrates to gypsy oth NPV. Grant No. 23-Final report. (Univ. of Connecticut, Biological Sciences Group, Ecology Section, U-42 for U.S. Forest Service; unpublished study; CDL:231360-M). MRID No. 00068408.

Sundaram, K.M.S. 1996. Leaching, mobility and persistence of tebufenozide in columns packed with forest litter II. J. Environmental Science and Health. B31(6): 1215-1239.

Surprenant, D. 1988. The chronic toxicity of carbon[14]-diflubenzuron to *Daphnia magna* under flow-through conditions: Laboratory Project ID: 11493.0188.6109.130. Unpublished study prepared by Springborn Life Sciences, Inc. MRID No. 40840501.

Surprenant, D. 1989. Acute toxicity of diflubenzuron to Quahogs (*Mercenaria mercenaria*) embryo-larvae Under Static Conditions. Lab Project Number: 89-3-2945: C 303.51.009. Unpublished study prepared by Springborn Laboratories, Inc. MRID No. 41392001.

Swank, W.T.; Waide, J.B.; Crossley, D.A., Jr.; Todd, R.L. 1981. Insect defoliation enhances nitrate export from forest ecosystems. Oecologia (Berlin) 51: 297-299.

Swift, M.C.; Smucker, R.A.; Cummins, K.W. 1988. Effects of Dimilin® on freshwater litter decomposition. Environmental Toxicology and Chemistry 7: 161-166.

T

Tanner, D.K.; Moffett, M.F. 1995. Effects of diflubenzuron on the reproductive success of the bluegill sunfish, *Lepomis macrochirus*. Environmental Toxicology and Chemistry 14(8): 1345-55.

Taylor, N., ed. 1961. Taylor's encyclopedia of gardening (horticulture and landscape design). 4th edition. Cambridge, MA: Houghton Mifflin Co.

Thorpe, K.W. 1996. Effects of ground-based applications of soap, *Bacillus thuringiensis*, cyfluthrin, and trunk barriers on gypsy moth density and defoliation. Journal of Arboriculture 22(2): 87-91.

Thorpe, K.W.; Webb, R.E.; Ridgway, R.L.; Venables, L.; Tatman, K.M. 1993. Sticky barrier bands affect density of gypsy moth (Lepidoptera: Lymantriidae) and damage in oak canopies. Journal of Economic Entomology 86(5): 1497-1501.

Thorpe, K.W.; Ridgway, Richard L. 1994. Effects of trunk barriers on larval gypsy moth (Lepidoptera: Lymantriidae) density in isolated- and contiguous-canopy oak trees. Environmental Entomology 23(4): 832-836.

Thorpe, K.W.; Podgwaite, J.D.; Slavicek, J.M.; Webb, R.E. 1998. Gypsy moth (Lepidoptera: Lymantriidae) control with ground-based hydraulic applications of Gypchek, *in vitro*-produced virus, and *Bacillus thuringiensis*. Journal of Economic Entomology 91(4): 875-880.

Thurber, D.K. 1993. Effects of gypsy moth-caused tree mortality on bird habitat. In: Fosbroke, S.L.C.; Gottschalk, K.W., editors. Proc., USDA Interagency gypsy Moth Research. Forum General Technical Report Cited in USDA 1995b.

Thurber, D.K.; McClain, W.R.; Whitmore, R.C. 1994. Indirect effects of gypsy moth defoliation on nest predation. Journal of Wildlife Management 58(3): 493-500.

Ticehurst, M.; Fursco, R.A.; Kling, R.P.; Unger, J. 1978. Observations on parasites of gypsy moth in first cycle infestations in Pennsylvania from 1974-1977. Environmental Entomology 7(3): 355-358.

Tigner, T. 1992. Gypsy moth impact of Virginia's hardwood forests and forest industry. Charlottesville, VA: Virginia Department of Forestry.

Tomblin, D.C. 1994. [Untitled]. Unpublished manuscript on file at Forestry Sciences Laboratory, West Virginia University, Morgantown, WV.

Tuthill, R.W.; Canada, A.T.; Wilcock, K.; Etkin, P.H.; O'Dell, T.M.; Shama, S.K. 1984. An epidemiologic study of gypsy moth rash. American Journal of Public Health 74(8): 799-803.

Twery, M.J. 1987. Changes in vertical distribution of xylem production in hardwoods defoliated by gypsy moth. New Haven, CT: Yale University, Ph.D. dissertation.

Twery, M.J. 1991. Effects of defoliation by gypsy moth. In: Gottschalk, Kurt W.; Twery, Mark J.; Smith, Shirley I., editors. Proceedings, U.S. Department of Agriculture Interagency Gypsy Moth Research Review; 1990 January 22-25; East Windsor, CT. General Technical Report NE-146. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station: 27-39.

Twery, M.J.; Gottschalk, K.W. 1988. Silviculture vs. the gypsy moth: can it help? In: Healthy forests, healthy world. Proceedings, Society of American Foresters National Convention; 1988 October 16-19; Rochester, NY. SAF Publication. 88-01. Bethesda, MD: Society of American Foresters: 169-172.

U

USDA (U.S. Department of Agriculture). 1985. Gypsy moth suppression and eradication projects: final environmental impact statement as supplemented—1985. Washington, DC: Forest Service, and Animal and Plant Health Inspection Service.

U.S. Department of Agriculture. 1989. Memorandum of understanding between the U.S. Department of Agriculture, Forest Service and the U.S. Department of Agriculture, Animal and Plant Health Inspection Service for management of the gypsy moth, October 1. Washington, DC.

U.S. Department of Agriculture.1990. Departmental gypsy moth policy. Department Regulation 5600-1. Washington, DC.

U.S. Department of Agriculture. 1992. Potential impacts of Asian gypsy moth in the western United States. Washington, DC and Hyattsville, MD: Forest Service in consultation with the Animal and Plant Health Inspection Service.

U.S. Department of Agriculture. 1993. USDA programs related to integrated pest management. Program Aid 1506. Washington, DC.

U.S. Department of Agriculture. 1995. Gypsy moth management in the United States: a cooperative approach: final environmental impact statement as supplemented. Washington, DC: Forest Service and Animal and Plant Health Inspection Service.

U.S. Department of Agriculture. 1996. Gypsy moth management in the United States: a cooperative approach. Record of Decision. Washington DC. USDA Forest Service and Animal and Plant Health Inspection Service.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service.1990. Gypsy moth program manual. Frederick, MD: Plant Protection and quarantine [pagination not continuous].

U.S. Department of Agriculture, Animal and Plant Health Inspection Service. 1992. Asian gypsy moth emergency program manual: Washington, D.C.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service. 2007. European gypsy moth, *Lymantria dispar*, quarantine. www.aphis.usda.gov/ppq/maps/gypmoth.pdf. (April 11, 2007)

U.S. Department of Agriculture, Forest Service. 1989. Final environmental impact statement for the Appalachian Integrated Pest Management (AIPM) Gypsy Moth Demonstration Project Management Bulletin R8-MB 33. Atlanta, GA: Southern Region, and Northeastern Area State and Private Forestry [pagination not continuous].

U.S. Department of Agriculture, Forest Service. 1990a. Forest Service Manual 2300-Recreation, wilderness, and related resource management, Amendment Number 2300-90-1, 1 June. Washington, DC.

U.S. Department of Agriculture, Forest Service. 1990b. Forest Service Manual 3400, Forest pest management. Amend. Number 3400-90-1, 1 June. Washington, DC.

U.S. Department of Agriculture, Forest Service. 1993. Urban forestry, fiscal year 1993: a status report. Radnor, PA; Northeastern Area State and Private Forestry.

U.S. Department of Agriculture, Forest Service. 1994a. Cooperative suppression and eradication projects: guidelines for participating state agencies. Radnor, PA; Northeastern Area State and Private Forestry [pagination not continuous].

U.S. Department of Agriculture, Forest Service. 1994b. Forest Service Manual 2300, Recreation, wilderness, and related resource management. Amendment Number 2300-94-1, 17 June. Washington, DC.

U.S. Department of Agriculture, Forest Service. 1994c. Forest Service Manual 4000, Research Amendment Number 4000-94-2, 4 May. Washington, DC.

U.S. Department of Agriculture, Forest Service. 1994d. Gypsy moth digest. Database maintained at Northeastern Area State and Private Forestry, Morgantown, WV.

U.S. Department of Agriculture, Forest Service. 1994e. The Appalachian Integrated Pest Management Gypsy Moth Project: final status report. Radnor, PA; Northeastern Area State and Private Forestry.

U.S. Department of Agriculture, Forest Service. 1994f. The gypsy moth in southern and eastern national forests: to treat or not to treat? A reference guide for forest managers. Atlanta, GA: Southern Region. Unpublished draft.

U.S. Department of Agriculture, Forest Service. 2003. Gypsy Moth Digest. http://na.fs.fed.us/fhp/gm/. [Date accessed unknown].

U.S. Environmental Protection Agency. 1975. DDT—a review of scientific and economic aspects of the decision to ban its use as a pesticide. EPA 540/1-785/022. Available from NTIS, Springfield, VA: PB-245029.

U.S. Environmental Protection Agency. 1987a. Recommendations for and documentation of biological values for use in risk assessment. ECAO-CIN-554. Cincinnati, OH: Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office [pagination not continuous].

U.S. Environmental Protection Agency. 1987b. The risk assessment guidelines of 1986. EPA 600/8-87/045. Washington, DC: Office of Health and Environmental Assessment, Research and Development [pagination not continuous]. Available from NTIS, Springfield, VA: PB-88-123997/AS.

U.S. Environmental Protection Agency. 1997. Re-registration Eligibility Decision (RED): Diflubenzuron. EPA 738-R-97-008. Office of Pesticide Programs.

U.S. Environmental Protection Agency. 1999a. Tebufenozide pesticide tolerances for emergency exemptions. Federal Register. 64(51): 13088-13094.

U.S. Environmental Protection Agency. 1999b. Tebufenozide Pesticide Tolerance Final Rule. Federal Register 64(183): 51251-51258.

U.S. Environmental Protection Agency. 1999c. Tebufenozide: Tier I Drinking Water EECs for use in the Human Health Risk Assessment. Memo from Dana Spatz and Jim Cowles dated January 19, 1999. (Copy courtesy of Janet Bressant, Public Information and Records Integrity Branch, U.S. EPA/OPP).

U.S. Environmental Protection Agency. 1999d. EFED risk assessment for Section 3 registration of tebufenozide (Confirm 2F and 70WSP) for use on tree nuts and almond hull; DP Barcode: D255779. Memo from N.E. Federoff, M. Rexrode, and J. Cowles dated July 13, 1999. (Copy courtesy of Janet Bressant, Public Information and Records Integrity Branch, U.S. EPA/OPP).

U.S. Environmental Protection Agency. 1999e. EFED risk assessment for Section 3 registration of tebufenozide (Confirm 2F and 70WSP). Memo from M. Rexrode, and J. Cowles dated March 1, 1999. (Copy courtesy of Janet Bressant, Public Information and Records Integrity Branch, U.S. EPA/OPP).

V

Valenti, M.A. 1998. *Entomophaga maimaiga*: salvation from the gypsy moth or fly in the ointment? American-Entomologist 1998, 44: 1, 20-22.

Valentine, B.J.; Gurr, G.M.; Thwaite, W.G. 1996. Efficacy of the insect growth regulators tebufenozide and fenoxycarb for Lepidopteran pest control in apples and Their Compatibility with Biological Control for Integrated Pest Management. Australian Journal of Experimental Agriculture 36 (4): 501-506.

Van der Kolk, J. 1997. RH-5992: Chronic Effects on Midge Larvae (*Chironomus riparius*) in a Water/Sediment System: Final Report: Lab Project Number: 95RC-0196: 96-047-1007: 1007.011.173. Unpublished study prepared by Spring. MRID No. 44198301.

Van Dersal, W.R. 1938. Native woody plants of the United States, their erosion-control and wildlife values. USDA Miscellaneous Publication 303. Washington, DC: U.S. Department of Agriculture.

Vaughan, M.R.: Kasbohm, J.W. 1993. Response of black bears to gypsy moth infestations in Shenandoah National Park, Virginia. In: Fosbroke, Sandra L.C.; Gottschalk, Kurt W., editors. Proceedings, U.S. Department of Agriculture Interagency Gypsy Moth Research Forum; 1993 January 19-22; Annapolis, MD. General Technical Report NE-179. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station.

Viertel, A.T. 1970. Trees, shrubs and vines (a pictorial guide to the ornamental woody plants of the northern United States, exclusive of conifers). Syracuse, NY: Syracuse University Press [no page numbers].

Vitousek, P.M.; Melillo, J.M. 1979. Nitrate losses from disturbed forests: patterns and mechanisms. Forest Science 25(4): 605-619.

W

Walgenbach, J.F. 1995. Effect of various insecticide programs on pest and beneficial arthropods in apples. Rohm and Hass Cooperator Report No. 2599521. Unpublished report; summarized in Keller 1998.

Wallner, W. 1992. A US/Canada chronicle. Gypsy Moth Exotica. Hamden, CT: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station; 4 p.

Wallner, W. 1994. Research planning and accomplishments. Gypsy Moth Exotica. Hamden, CT: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station; 1-2.

Wargo, P.M. 1978a. Defoliation by the gypsy moth: how it hurts your tree. Home and Garden Bulletin 223. Washington, DC: U.S. Department of Agriculture.

Wargo, P.M. 1978b. Insects have defoliated my tree—now what's going to happen? Journal of Arboriculture 4(8): 169-175.

Wargo, P.M. 1981a. Defoliation and tree growth. In: Doane, Charles C.; McManus, Michael L., editors. The gypsy moth: research toward integrated pest management. Technical Bulletin 1584. Washington, DC: U.S. Department of Agriculture: 225-240.

Wargo, P.M. 1981b. Defoliation, dieback, and mortality. In: Doane, Charles C.; McManus, Michael L., editors. The gypsy moth: research toward integrated pest management. Technical Bulletin 1584. Washington, DC: U.S. Department of Agriculture: 240-248.

Webb, R.E.; Boyd, V.K. 1983. Evaluation of barrier bands and insecticidal strips for impeding intraplant movement of gypsy moth caterpillars. Melscheimer Entomological Series 33: 15-20.

Webb, R.E.; Shapiro, M.; Podgwaite, J.D.; Reardon, R.C.; Tatman, K.M.; Venables, L.; Kolodny-Hirsch, D.M. 1989. Effect of aerial spraying with Dimilin®, Dipel, or Gypchek on two natural enemies of the gypsy moth (Lepidoptera: Lymantriidae). Journal of Economic Entomology 82(6): 1695-1701.

Webb, J.R.; Cosby, B.J.; Deviney, F.A., Jr.; Eshleman, K.N.; Galloway, J.N. 1995a. Change in the acid-base status of an Appalachian mountain catchment following forest defoliation by the gypsy moth. Proceedings from the 5th International Conference on Acidic Deposition: Science and Policy, Goteborg, Sweden, 26-30 June, 1995. Water Air and Soil Pollution 85(2): 535-540.

Webb, R.E.; White, G.B.; Thorpe, K.W. 1995b. Response of gypsy moth (Lepidoptera: Lymantriidae) larvae to sticky barrier bands on simulated trees. Proceedings of the Entomological Society of Washington 97(3): 695-700.

Webb, R.E.; White, G.B.; Thorpe, K.W. 2003a. Augmenting nucleopolyhedrovirus load in gypsy moth (Lepidoptera: Lymantriidae) populations with egg mass treatments. Journal of Entomological Science 38(2): 300-313.

Webb, R.E.; White, G.B.; Thorpe, K.W. 2003b. Low incidence of viral and fungal entomopathogens in gypsy moth (Lepidoptera: Lymantriidae) populations in Northern Virginia. Journal of Entomological Science 38(2): 314-316.

Weiss, M.J.; Rizzo, D.M. 1987. Forest declines in major forest types of the eastern United States. In: Kairiukstis, L; Nilsson, S.; Straszak, A., editors. Proceeding of the workshop, Forest decline and reproduction: regional and global consequences; 1987 March 23-27; Krakow, Poland. WP-87-75. Laxenburg, Austria: International Institute for Applied Systems Analysis: 297-305.

Weseloh, R.M. 1976. Reduced effectiveness of gypsy moth parasite, *Apanteles melanoscelus*, in Connecticut due to poor seasonal synchronization with its host. Environmental Entomology 55 (4): 743-746.

Weseloh, R.M. 1983. Population sampling method of cocoons of the gypsy moth (Lepidoptera: Lymantriidae) parasite, *Apanteles melanoscelus* (Hymenoptera: Braconidae), and relationship of its population levels to predator-and hyperparasitic-induced morality. Environmental Entomology 12(4): 1228-1231.

Weseloh, R.M. 1985a. Changes in population size, dispersal behavior, and reproduction of *Calosoma sycophanta* (Coleoptera: Carabidae), associated with changes in gypsy moth, Lymantria (Lepidoptera: Lymantriidae) abundance. Environmental Entomology 14(3): 370-377.

Weseloh, R.M. 1985b. Predation by *Calosoma sycophanta* L. (Coleoptera: Carabidae): evidence for a large impact of gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae), pupae. The Canadian Entomologist 117(9): 1117-1126.

Weseloh, R.M. 1988. Effects of microhabitat, time of day, and weather on predation of gypsy moth larvae. Oecologia 77(2): 250-254.

Weseloh, R.M. 1998a. Modeling the influence of forest characteristics and ant (Formicidae: Hymenoptera) predation on dispersal and survival of neonate gypsy moths (Lymantriidae: Lepidoptera). Environmental Entomology 27(2): 288-296.

Weseloh, R.M. 1998b. Possibility for recent origin of the gypsy moth (Lepidoptera: Lymantriidae) fungal pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) in North America. Environmental Entomology 27(2): 171-177.

Weseloh, R.M. 2003a. Short and long range dispersal in the gypsy moth (Lepidoptera: Lymantriidae) fungal pathogen, *Entomophaga maimaiga* (Zygomycetes: Entomophthorales). Environmental Entomology 32(1): 111-122.

Weseloh, R.M. 2003b. A computer model of the gypsy moth and its fungal pathogen. Bulletin Connecticut Agricultural-Experiment-Station No. 987.

Weseloh, Ronald M. 2003c. People and the gypsy moth: a story of human interactions with an invasive species. American Entomologist 49 (3): 180-190.

Weseloh, R.M.; Andreadis, T.G. 1982. Possible mechanism for synergism between *Bacillus thuringiensis* and the gypsy moth (Lepidoptera: Lymantriidae) parasitoid. *Apanteles melanoscelus* (Hymenoptera: Braconidae). Annals of the Entomological Society of America 7(4): 435-438.

Weseloh, R.M.; Andreadis, T.G. 1997. Persistence of resting spores of *Entomophaga maimaiga*, a fungal pathogen of the gypsy moth, *Lymantria dispar*. Journal of Invertebrate Pathology 69(2): 195-196.

Weseloh, R.M.; Andreadis, T.G. 2002. Detecting the titer in forest soils of spores of the gypsy moth (Lepidoptera: Lymantriidae) fungal pathogen, *Entomophaga maimaiga* (Zygomycetes: Entomophthorales). Canadian Entomologist 134(2): 269-279.

Whelan, C.J.; Holmes, R.T.; Smith, H.R. 1989. Bird predation on gypsy moth (Lepidoptera: Lymantriidae) larvae: an aviary study. Environmental Entomology 18(1): 43-45.

White, G. B.; Webb, R. E; Thorpe, K. W.; Douglass, L.W. 1997. Evaluation of an insecticidal latex coating for control of late-stage gypsy moth larvae. Arthropod Management Tests 24:385.

Whitmore, R.C.; Cooper, R.J.; Sample, B.E. 1993. Bird fat-reductions in forests treated with Dimilin®. Environmental Toxicology and Chemistry 12: 2059-2064.

Williams, D.W.; Liebhold, A.M. 1995a. Influence of weather on the synchrony of gypsy moth (Lepidoptera: Lymantriidae) outbreaks in New England. Environmental Entomology 24(5): 987-995.

Williams, D.W.; Liebhold, A.M. 1995b. Forest defoliators and climatic change: potential changes in spatial distribution of outbreaks of western spruce budworm (Lepidoptera: Tortricidae) and gypsy moth (Lepidoptera: Lymantriidae). Environmental Entomology 24(1): 1-9.

Williams, D.W.; Liebhold, A.M. 1995c. Forest defoliators and climate change: potential changes in spatial distribution of outbreaks of western spruce budworm (Lepidoptera: Torticidae) and gypsy moth (Lepidoptera: Lymantriidae). Environmental Entomology 24(1): 1-9.

Wirth, S.; Vogel, K. 1988. Cow milk protein intolerance in infants with methemoglobinemia and diarrhea. European Journal of Pediatrics (Berlin) 148(2):172.

Woods, S.A.; Elkinton, J.S.; Shapiro, M. 1990. Factors affecting the distribution of nuclear polyhedrosis virus among gypsy moth (Lepidoptera: Lymantriidae) egg masses and larvae. Environmental Entomology 19(5): 1330-1337.

Work, T.T.; McCullough, D.G. 2000. Lepidopteran communities in two forest ecosystems during the first gypsy moth outbreaks in Northern Michigan. Environmental Entomology 29(5): 884-900.

Y

Yu, S.J.; Robinson, F.A.; Nation, J.L.1984. Detoxication capacity in the honey bee, *Apis mellifera*. Pesticide Biochemistry and Physiology. 22: 360-368. Summarized in WHO 1996.

Z

Zacarias, M.S.; de Moraes, J.C.; de Castro Diniz, L.; Ciociola, A.I.; Damasceno, A.G. 1998. Selectivity of insect growth regulators to eggs and nymphs of *Podisus nigrispinus* (Dallas) (Homoptera: Pentatomidae). Ciênc. E. Agrotec. Lavras 22(2): 194-198.

Zungoli, P.A.; Steinhauer, A.L.; Linduska, J.J. 1983. Evaluation of diflubenzuron for Mexican bean beetle (Coleoptera: Coccinellidae) control and impact on *Pediobius foveolatus* (Hymenoptera: Eulophidae). Journal of Economic Entomology 76: 188-191.

Pesticide Precautionary Statement

This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife--if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

Appendix A
Gypsy Moth Treatments and Application Technology

Figure A-1. *Public notices warned campers about transporting gypsy moth eggs and caterpillars.*

Appendix A Gypsy Moth Treatments and Application Technology

Contents

Figures

This appendix describes treatments used and proposed for use in managing the gypsy moth. These treatments vary in effectiveness in different situations. Some are not effective in meeting the objectives of eradication, suppression, or slow-the-spread projects; but they are presented in order to provide the reader with a fuller understanding of the range of control and natural agents that regulate gypsy moth populations.

The treatments are divided into four categories. The first category includes those treatments in the 1996 Record of Decision: *Bacillus thuringiensis* var. *kurstaki (B.t.k)*, diflubenzuron, the gypsy moth nucleopolyhedrosis virus product (Gypchek), mating disruption, mass trapping, and sterile insect technique. The second treatment category consists of the new proposed treatment of tebufenozide. The environmental and human health risks associated with the use of treatments in these first two categories are analyzed and presented in Appendixes F-K of this supplemental environmental impact statement (SEIS). The environmental effects are summarized in Chapter 4.

The third category contains some natural control agents that help regulate gypsy moth populations in North America and in other places around the world where gypsy moth exists. These natural control agents include fungal pathogens, parasitoids, predators, nematodes, and microsporidia. Unfortunately, cost effective technology does not yet exist to develop and propagate these agents for use within the USDA gypsy moth management program.

The fourth category contains the miscellaneous treatments of removing and destroying egg masses, tree trunk bands and barriers, broad-spectrum insecticides, and silviculture. These treatment methods do not meet the objectives of eradication, suppression, and slow-the-spread projects. Some of the treatments may have value, however, for protecting individual trees in homeowner's yards or other landscape situations, rather than in a forest setting or in a large treatment area.

A.1 Treatments in the 1996 Record of Decision.

Bacillus thuringiensis var. *kurstaki* (*B.t.k.*).

Bacillus thuringiensis, commonly called *B.t.*, is a bacterium that moves by using whip-like appendages called flagella and forms a resting spore. *B.t.* occurs naturally in soils throughout the world. Unique to this species is formation of a protein crystal next to the spore at the time of sporulation.

B.t. commercial formulations used for managing defoliating forest caterpillars in North America are preparations of the HD-1 strain of *B.t.* variety *kurstaki* (*B.t.k.*). *B.t.k.* spores and crystals are ingested by the gypsy moth caterpillar along with foliage. Enzymes in the mid-gut of the caterpillar dissolve the crystals and release delta-endotoxins, which are insecticidal crystal proteins. The proteins bind to specific receptors on the cellular lining of the midgut and penetrate the cell membrane. The insect stops feeding and dies within a few hours or days.

Natural epizootics caused by *B.t.k.* have not been observed as a control factor for the gypsy moth (Reardon and others 1994). *B.t.k.* is not expected to infect more than the current year generation of gypsy moths present when it is applied (Dubois and others 1988).

B.t.k. Use.

A number of commercial preparations of *B.t.k.* are registered for aerial and ground application to gypsy moth populations. The typical application rate used in USDA cooperative suppression projects is one application at 24 to 38 BIU per acre (60-95 BIU/ha). For eradication treatments, the typical dose rate is 24 to 25 BIU per acre (60-63 BIU/ha), applied one to three times with application times being from a few days to over a week apart.

The United States Department of Agriculture (USDA) first used *B.t.k.* in cooperative suppression projects for the gypsy moth in 1980. Between 1995 and 2003, *B.t.k.* was used in more than 68 percent of the total acreage treated in cooperative suppression projects, more than 1.4 million acres (0.5 million ha) in nine States (U.S. Department of Agriculture, Forest Service 2003).

The timing of *B.t.k.* application in gypsy moth projects is generally dictated by foliage and insect development (Dubois 1991). The optimal timing of application is when most of the insects are in the second instar, and not delayed beyond early third instar. To be effective, *B.t.k.* must be consumed by the caterpillar. The timing of *B.t.k.* application is a subjective judgment considering foliage expansion, larval stage, population density and predicted level of defoliation (Reardon and others 1994).

Phenology models such as BIOSIM may be used to help predict insect development in eradication projects where gypsy moth numbers are so low that egg masses and larvae cannot be located and monitored. This allows application of *B.t.k.* at the most opportune time. Caged egg masses are sometimes deployed in treatment areas and monitored for egg hatch so that the optimal timing of *B.t.k.* application can be estimated.

B.t.k. Effectiveness.

The effectiveness of *B.t.k.* in cooperative suppression projects from 2000 to 2003 varied from a low of 84 percent to a high of 100 percent; the average success rate of suppression projects was 95 percent in reducing gypsy moth populations (U.S. Department of Agriculture, Forest Service 2003). Greater reductions in gypsy moth populations generally occurred with higher dose rates (24 and 38 BIU per acre: 60 and 95 BIU/ha) (U.S. Department of Agriculture, Forest Service 2003).

Many factors affect *B.t.k.* efficacy, including the timing of the application with regard to insect and foliage development, weather conditions during and after application, and the quality of the application, that is, good pilot skills and properly functioning equipment. Most important is application timing and delivering a dose sufficient to kill the insects. The species of host plant may also affect the effectiveness of *B.t.k.* (Farrar and others 1996).

During eradication applications in or near areas that contain rare, endangered, or desirable moths and butterflies, extra effort should be taken to minimize drift (see Advances in Application Technology later in this appendix). See Appendix F for the risk assessment on *B.t.k.*

Diflubenzuron.

Diflubenzuron belongs to a group of compounds called insect growth regulators. When ingested by gypsy moth caterpillars, diflubenzuron disrupts the formation of a new cuticle (outer skin) during molting. The caterpillar cannot complete the molting process, its body wall ruptures from internal pressure, and the insect dies. Ingestion of diflubenzuron is lethal to the gypsy moth caterpillar.

Diflubenzuron Use.

Diflubenzuron is registered for aerial or ground application for gypsy moth. The label prohibits application directly to water, to areas where surface water is present, or to intertidal areas below the high water mark—except under the forest canopy when aerially applied. Typically, diflubenzuron is aerially applied at the rate of 0.5 ounces active ingredient in 0.75 to 1.00 gallon spray volume-per-acre, twice in eradication projects and once in suppression projects. Diflubenzuron application in suppression projects may be at a much lower dosage than the commonly used 0.5 ounce active ingredient per acre and still achieve project objectives (McLane 1993).

Diflubenzuron Effectiveness.

Diflubenzuron effectively reduces gypsy moth populations and protects foliage, both key objectives of suppression projects. Data collected from 2000 to 2003 from areas treated with diflubenzuron in cooperative suppression projects with States reveal diflubenzuron has a 95 to 98 percent success rate in meeting foliage protection objectives. From 1995 to 2003, diflubenzuron was used on about 30 percent of the total acres treated in cooperative suppression projects (GM Digest 2004). See Appendix I for the risk assessment on diflubenzuron.

Gypchek (Nucleopolyhedrosis Virus).

The gypsy moth nucleopolyhedrosis virus (NPV) is one of several natural agents found in eastern North America that infect gypsy moth (Podgwaite and Campbell 1972). The virus is a member of the genus *Baculovirus* and is unrelated to arthropod-borne viruses and other viruses that infect man (Mazzone and others 1976). The disease caused by the gypsy moth virus is commonly referred to as "wilt disease" because of the limp appearance of infected caterpillars.

The disease can reach outbreak levels naturally as gypsy moth populations increase. Epizootics caused by the gypsy moth virus are thought to be density dependent, and display one or more waves of mortality; intensity is proportional to larval density and viral inoculum (Doane 1970, Woods and others 1990). Outbreaks of this type result from increased transmission rates of the virus within and between generations of the gypsy moth. Small gypsy moth caterpillars become infected and die on leaves in the tree crowns, the cadavers disintegrate, and the viral particles disperse, infecting other gypsy moth caterpillars.

The virus appears to spread rather easily when egg masses are laid on virus-contaminated surfaces. Birds, mammals, gypsy moth parasitoids, and invertebrate predators may also play a role in spreading the virus, although they themselves are not affected. The virus

may kill up to 90 percent of the caterpillars in dense gypsy moth populations, reducing populations to levels that cause only minimal defoliation the following year (Reardon and Podgwaite 1992, Reardon and others 1996).

USDA began investigating the feasibility of developing gypsy moth virus as an alternative to chemical insecticides in the late 1950s. The viral product Gypchek was registered with the U.S. Environmental Protection Agency (U.S. EPA) in 1978 as a general use insecticide for ground and aerial application (Reardon and Podgwaite 1992, Reardon and others 1996). Gypchek must be used under the supervision of the Forest Service.

Gypchek is specific to the gypsy moth and does not affect other caterpillar species or any other nontarget organisms that might be present in treatment areas (Barber and others 1993, Rastall and others 2003). This fact renders Gypchek a desirable insecticide for use where threatened or endangered species might be found or in other environmentally sensitive areas; however, the availability of Gypchek is limited.

Gypchek is produced by the Forest Service and APHIS in quantities sufficient to treat about 8,000 acres (3,240 ha) each year. Production involves raising large numbers of gypsy moth caterpillars, inoculating and then processing the infected caterpillars at the appropriate time. Anywhere from 500 to 1,000 infected caterpillars are required to produce enough Gypchek to treat 1 acre with two applications. Widespread operational use of Gypchek hinges on availability and cost (Reardon and Podgwaite 1992,Reardon and others 1996). Gypchek can be applied with aerial or ground techniques, and when applied properly can achieve suppression rates similar to *B.t.k.* (Thorpe and others 1998).

On-going research may result in the future ability to manufacture Gypchek in bioreactors, avoiding the higher costs and difficulty of rearing caterpillars to

produce the virus *(Figure A-2).* Research also seeks to produce a strain of Gypchek that is more effective against the gypsy moth.

Gypchek must be ingested by the gypsy moth caterpillar. The rod-shaped virus particles, or virions, are liberated in the gut of the insect. The virions invade the gut wall and attack the internal organs and tissues, causing infection. The virus multiplies rapidly in cells of the insect and eventually causes breakdown of internal tissue and death. The entire process takes from 10 to 14 days, depending on the size of the caterpillar, viral dose, and ambient temperature. First and second instar caterpillars are most susceptible to Gypchek. Dead caterpillars typically hang in an inverted "V" from foliage and branches and often rupture, releasing more virus that can infect other gypsy moths (Reardon and Podgwaite 1992, Reardon and others 1996) (*Figure A-3*).

Gypchek Use.

Gypchek must be formulated at the mixing and loading site before aerial application. The standard tank mix consists of water (pH 5.0-8.0), ultraviolet-light sunscreen and a sticking agent (to aid adhesion to leaf surfaces). During the years 1995 to 2003, Gypchek was used on an average of 5,014 acres per year for suppression, eradication, and slow-the-spread treatments (U.S. Department of Agriculture, Forest Service 2003). Gypchek is usually applied against first or second instars of the gypsy moth.

Gypchek Effectiveness.

Gypchek is preferably used against moderate-to-high gypsy moth populations (300-5,000 egg masses/acre [741-12,355 egg masses/ha]). Gypchek does not adversely affect nontarget species (Rastall and others 2003). See Appendix G for the risk assessment on Gypchek.
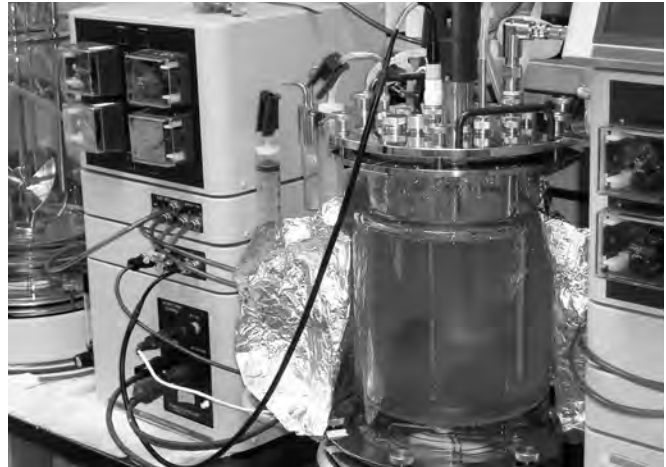
Figure A-2. Researchers hope to be able to manufacture Gypchek in bioreactors such as this. (Forest Service laboratory, Delaware, OH)
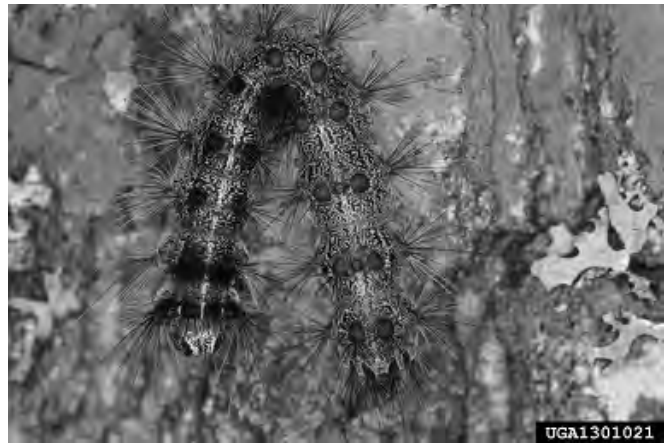
Figure A-3. Gypsy moth larvae killed by the nucleopolyhedrosis virus typically hang in an inverted V.

Mass Trapping (Disparlure Only, or Disparlure and Dichlorvos).

Mass trapping uses disparlure (synthetic sex pheromone) to attract male moths to traps placed in a grid pattern across a target area, with the objective of capturing male gypsy moths before they are able to locate and mate with female moths. Two types of traps are used, depending on the expected number of moths to be caught: the smaller delta trap and larger milk carton trap.

The standard "delta" trap is a small-capacity trap, approximately 8 inches (20 cm) long, 4 inches (10 cm) high, and triangular in cross section. A tiny plastic strip impregnated with disparlure or a string impregnated with disparlure is stapled to the inside of the trap to attract male gypsy moths. The inside surface of the trap is coated with a sticky substance to capture the moths and prevent their escape from the traps.

The second type of trap is called the "milk carton" trap because it resembles a half-gallon cardboard milk container. This type of trap is used in areas where large numbers of male moths are expected to be caught and would quickly overwhelm the sticky surface of the smaller delta trap. As in the delta trap, a small pheromone wick containing disparlure is placed inside the milk carton trap to attract moths. Unlike the delta trap, the milk carton trap also contains a 1-inch by 4-inch (2.5 cm by 10 cm) laminated plastic strip impregnated with the insecticide dichlorvos (2,3 dichloroethenyl dimethyl ester phosphoric acid [DDVP]) to kill the moths and prevent their escape from the traps. Dichlorvos, registered with the U.S. EPA, is manufactured by AMVAC Chemical Corporation (City of Commerce, CA). When used in milk carton traps, dichlorvos is formulated and registered as Vaportape II (Hercon Environmental Company, Emigsville, PA). A risk analysis for dichlorvos is found in Appendix K of this SEIS.

Mass Trapping Use.

Both types of traps are used for detecting and monitoring gypsy moth populations. Delta traps are most commonly used in the uninfested area of the United States to detect and delimit isolated infestations of the gypsy moth. Milk carton traps are more commonly used in areas where large numbers of male moths are likely to be caught. The delta trap is primarily used for mass trapping, though milk carton traps might be considered if the expected catch per trap is greater than 15 moths, which would overwhelm the sticky surface in the smaller delta trap. When used for mass trapping, delta or milk carton traps are deployed in an intensive grid pattern across an infested area and an adjacent buffer area, at the rate of at least 9 traps per acre (25 traps/ha).

Mass Trapping Effectiveness.

The success of mass trapping depends on the density of the gypsy moth population in the treatment area, because the tactic relies on luring all male moths into the traps before they can mate with females. The higher the population density, the greater the risk that a male will find and mate with a female before being lured into a trap. Therefore, the treatment is best used where there are less than 10 egg masses per acre (25 egg masses/ha) (USDA Forest Service 1989).

Mass trapping is a labor-intensive treatment, especially over large areas; it is typically used on small infestations of less than 100 acres (40.4 ha). Nontarget organisms are unaffected, except those that accidentally find their way into the traps (primarily flying insects).

See Appendix H for the risk assessment on disparlure, and Appendix K for the risk assessment on dichlorvos.

Mating Disruption (Disparlure).

Disparlure is a gypsy moth pheromone that attracts male moths to female moths for mating. Synthetically produced disparlure can be used to disrupt the mating of gypsy moths. Mating disruption relies on the use of the gypsy moth pheromone disparlure (*cis*-7,8-epoxy-2-methyloctadecane [racemic disparlure]) as the active ingredient; however, a 50:50 mixture of the plus (+) and minus (−) enantiomers of synthetic disparlure are used rather than only the plus (+) enantiomer used in trap lures. This 50:50 mixture of enantiomers, called racemic disparlure, lacks the highly attractive characteristics of plus disparlure. Instead of luring adult male gypsy moths away from females, application of racemic disparlure interferes, or "disrupts," the male moths' normal mate search behavior, which prevents them from finding and mating with the females.

Mating Disruption Use.

Mating disruption was first used operationally in a USDA cooperative eradication project in Virginia in 1983. Widespread use of this treatment did not begin until initiation of the slow-the-spread pilot project in 1993. Research and technology development accelerated during the pilot project (1993 to 1999). By the time slow the spread transitioned to operational status in 2000, mating disruption evolved into the treatment of choice. Between 2000 and 2004, mating disruption accounted for more than 84 percent of the total acres treated in association with slow-the-spread projects. During that period, a total of 1.7 million acres in the slow-the-spread area received treatment, with 1.4 million acres receiving mating disruption treatments. Only 15 uses of mating disruption as the primary treatment are recorded for eradication projects between 1983 and 2003. This represents less than 2 percent of the total acreage treated for eradication during that period (U.S. Department of Agriculture, Forest Service 2003).

Mating disruption is accomplished either by ground or aerial application of controlled-release dispensers, formulated to slowly exude their load of active ingredient (racemic disparlure) into the environment. The formulation used for ground application consists of a laminated polymeric dispenser or tape impregnated with the pheromone for gradual release into the environment (USDA Forest Service 1989). The tape is manually attached to trees in a grid pattern, making this method labor intensive, especially in large treatment areas. An evaluation of this method concluded that additional research is needed before considering it for operational use (Kolodny-Hirsch and others 1990). The tape is no longer produced, but it is still registered with the U.S. EPA and could be made available in the future if requested from the manufacturer (Hercon Environmental Co, Emigsville, PA).

Although numerous controlled-release dispensers have been evaluated for use in aerial gypsy moth mating disruption projects, only Hercon's Disrupt II is registered with EPA and available for commercial use. This formulation consists of a layer of resin impregnated with racemic disparlure sandwiched between two outer layers of plastic laminate. The laminate is chopped into small flakes; thus the commonly used term "pheromone flakes" or simply "flakes" when referring to Disrupt II treatments. Aircraft using custom-designed application equipment apply the flakes, which slowly release their load of pheromone into the environment over 3 to 4 months. Other promising formulations, such as microcapsules, microtubes, or emulsified concentrates, will continue to be evaluated for use in gypsy moth mating disruption projects.

Mating Disruption Effectiveness.

The effectiveness of mating disruption varies with the population density of gypsy moths in the treatment and surrounding areas. Mating disruption is only effective when used against very low-density populations of the gypsy moth; in higher-density populations where dozens of moths of both sexes may emerge on the same tree bole, the chance of male moths locating females is high. Therefore, mating disruption is best suited for areas that contain less than 10 egg masses per acre (25 egg masses/ha) (USDA Forest Service 1989). Population densities of this sort are typically found in the STS or eradication area, but not in the suppression area.

The trend in recent years has been toward lower doses for gypsy moth slow-the-spread projects. Until 1999, the standard dose used in slow-the-spread mating disruption projects was 30 grams active ingredient per acre (30 g a.i. per acre). Reduction of the standard dose by 50 percent started in 2000, to 15 g a.i. per acre. Further research confirms doses as low as 6 g a.i. per acre can effectively disrupt mating in low-density populations of the gypsy moth.

Mating disruption may be used alone or in conjunction with other treatments. Typically, it is used alone, but in some situations large infestations contain core

population(s) that already exceed the threshold at which mating disruption can be effective. In these cases, a small area treated with *B.t.k.*, diflubenzuron, or Gypchek might be embedded within the boundaries of the larger mating disruption block.

Treatments using mating disruption as part of cooperative slow-the-spread projects between 1993 and 2001, were at least as effective as treatments using *B.t.k.* Further, the frequency of repeated treatments was higher after using *B.t.k.* than mating disruption. This information must be further evaluated, considering that mating disruption is typically used on the lower population densities, whereas *B.t.k.* is used on both low and high population densities (Sharov and others 2002a).

The use of disparlure as a mating disruption agent is desirable because the pheromone does not affect nontarget organisms. Once the pheromone dissipates, however, the plastic dispensers may remain in the environment for some time before disintegrating. Nonetheless, the use of this treatment will continue to be critical to STS projects where rare, threatened, or endangered species arecommonly encountered.

See Appendix H for the risk assessment on disparlure.

Sterile Insect Technique.

The sterile insect technique has not been used in recent years, but is available as a treatment tool for gypsy moth control. The objective of the sterile insect technique is to reduce the chance that female moths will mate with fertile males. Its success is more likely with the release of large numbers of sterile males in consecutive years. The resultant progressive reduction of fertile egg mass production leads to the eventual elimination of the population.

Sterile insect technique is ideally suited for application to gypsy moth populations with one generation per year. Male moths may mate several times; female moths usually mate only once and lay an egg mass that may contain up to 1,600 eggs (Reardon and Mastro 1993). Recognition of the potential of this approach for managing low-density and isolated infestations of the gypsy moth took place in the mid-1950s. Treatment was not practical, however, until the development of methodologies for rearing large quantities of quality insects and quantifying the impact of the releases (Mastro and others 1981).

Sterile Insect Technique Use.

One of three different approaches is selected (Reardon and Mastro 1993): (1) deploying male pupae sterilized by irradiation; (2) deploying male pupae irradiated, but not fully sterilized (substerile); or (3) broadcasting eggs from a female mated with an irradiated male (inherited sterility). None of these approaches is without biological or logistical limitations, which hamper operational use.

Sterile and Substerile Male Pupae.
Initially, the sterile insect technique focused on deploying male pupae treated with a sterilizing dose of radiation. Pilot projects in Maryland, Michigan, and South Carolina during the 1970s and 1980s demonstrated the efficacy of this technique. Nevertheless, the limited time period during which pupae must be released and the need to synchronize rearing of mass quantities of pupae for that release (treated pupae cannot be stockpiled) are obstacles to an operational program *(Figure A-4)* (Reardon and Mastro 1993). A major logistical difficulty is the necessity of repeatedly releasing the treated insects over the 4-week flight period because male moths live only 2 to 3 days.

Deploying substerile insects is the preferred of the two techniques that release male pupae, because (1) the substerile insects suffer less tissue damage and are therefore more competitive than sterile males; (2) the progeny of substerile males and wild females develop in the field and are, in theory, hardy and in synchrony

Figure A-4. Sterile gypsy moths are reared on artificial diet in a climate-controlled environmental chamber.

with the native population; and (3) the suppressive effect on the native population spans at least two life cycles (Knipling 1979, Snow and others 1971).

Inherited Sterility.

n induced inherited sterility (or F1 sterility), males are irradiated but not sterilized before they mate with non-irradiated females in the laboratory. More of the resulting progeny are sterile than in the treated parental generation, and the sex-ratio of the progeny is skewed in favor of males (LaChance 1985, North 1975). Release of F1 sterile eggs has advantages over the other two techniques: only a single release of treated gypsy moth eggs is required before wild eggs hatch,

the production window is wider because eggs can be stockpiled, and the logistics of shipment and release are simpler.

Sterile Insect Technique Effectiveness.

Between 1988 and 1992, eight isolated infestations of the gypsy moth were treated by releasing F1 sterile eggs, with favorable results; but numerous problems were identified (Reardon and Mastro 1993): (1) how to predict when wild eggs hatch, and how to synchronize release and hatching of eggs produced in the laboratory; (2) how to reduce mortality that occurs in early F1 instars; (3) dispersal of F1 young caterpillars and adult males; and (4) the relative competitiveness of caterpillars.

When evaluated against low-level gypsy moth populations in Virginia (Reardon 1991), results with substerile pupae generally proved more favorable than with F1 sterile eggs. Of the three approaches, the deployment of sterile male pupae is the least desirable. Release of F1 sterile eggs is preferred; however, the obstacles described are major impediments to more general use of this technique (Reardon and Mastro 1993). The deployment of substerile pupae, in spite of its disadvantages, appears closest to operational use, although availability of substerile insects is limited.

Recent advances in insect engineering for use in sterile insect technique programs show promise for increasing the effectiveness and efficiency of the program. At this writing, however, no operational program has been developed for the gypsy moth.

A.2 The New Proposed Treatment of Tebufenozide.

Tebufenozide, like diflubenzuron, belongs to a group of compounds called insect growth regulators. Tebufenozide, which induces premature molts by direct stimulation of the ecdysteroid receptors (whereas diflubenzuron affects chitin synthesis at the regularly scheduled molt), mimics the action of a natural insect

hormone. Upon ingestion of tebufenozide, larvae stop feeding and undergo an early, incomplete and lethal molt.

Tebufenozide Use.

Label instructions permit ground or aerial applications of tebufenozide. The labeled application rates for tebufenozide range from 0.06 lbs a.i. per acre to 0.12 lbs a.i. per acre. Tebufenozide is applied to early first to third instar larvae.

Tebufenozide Effectiveness.

Tebufenozide has not been used operationally by the USDA in suppression, eradication, or slow-the-spread projects. Forest Service tests of tebufenozide at 0.06 lbs a.i. per acre generally found the product to be effective with 95 to 99 percent control achieved (Reardon and others 2000). See Appendix J for the risk assessment on tebufenozide.

A.3 Treatments That Include Natural Control Agents.

Few natural control agents accompanied the accidental introduction of the gypsy moth to this country. Some of those agents, as well as agents native to the United States, can play an important role in regulating gypsy moth populations throughout the generally infested area.

Fungal Pathogens.

Fungal products labeled for use against the gypsy moth are not available at this writing. A fungus capable of infecting the gypsy moth is *Entomophaga maimaiga* Humber, Shimazu, and Soper. This fungus, commonly found in Japan (Soper and others 1988), was brought to the United States in the early 1900s and released, but was not recovered until 1989 (Hajek and others 1996). *E. maimaiga* is known to infect only the gypsy moth and other closely related caterpillars that spend significant periods of time on the soil surface (Reardon and Hajek 1993, Hajek and others 2000).

A field survey, conducted from 1989 to 1995 of lepidopteran cadavers infected with *E. maimaiga*, found three species of lymantriids, from the genus *Dasychira*, infected with *E. maimaiga* (Hajek and others 1996b). The field survey method was chosen because entomopathogens can infect hosts in the laboratory that are never found infected in the field. During a similar study in 1994, Hajek and others tested and found two species infected with *E. maimaiga*. Under laboratory conditions, conidia (infective spores) produced by an alternate host were determined to be ineffective (Hajek and others 1995b).

Epizootics of *E. maimaiga* in gypsy moth identified in the northeastern United States in 1989 represent the first reported occurrence of this fungus in North American gypsy moth populations (Andreadis and Weseloh 1990, Hajek and others 1990). Unlike the gypsy moth nucleopolyhedrosis virus, associated with high gypsy moth population densities, the fungus appears capable of causing dramatic mortality to middle-and late-stage gypsy moth caterpillars at low densities (Shimazu and Soper 1986). Since the fungus tends to cause mortality earlier than the virus, tree defoliation may not be as severe. Prediction of long-term impacts of *E. maimaiga* is inconclusive (Valenti 1998).

A gypsy moth larvae infected with *E. maimaiga* produces one or both types of spores—resting spores (azygospores) and conidia spores (infectious spores).

The age of the larvae is the primary factor in determining which type of spore is produced. Second instar larvae rarely contain resting spores, while fifth instar larvae produce resting spores when temperatures are increasing (Hajek and Shimazu 1996). The resting spore of the fungus overwinters on the bark of trees, in leaf litter, and in soil (Shimazu and others 1986). The resting spore germinates in the spring and produces a single conidium, which is released into the environment and may be carried in the air. Once on a susceptible caterpillar, the conidium germinates, penetrates the

insect's skin, spreads throughout the caterpillar and kills it. Infected dying caterpillars typically hang with head down, in a stretched-out position on the stems of infested trees (Hajek and Roberts 1992). The fungal spores may remain alive in the soil for up to 10 years (Weseloh and Andreadis 2002).

With favorable conditions, high humidity and temperatures between 13 °C to 19 °C, the fungus grows out of the caterpillar through the skin and produces and releases more conidia, which may subsequently infect other caterpillars (Hajek and others 1996c). This secondary infection cycle is a major contributor to the dramatic epizootics observed in gypsy moth populations. When conditions are unfavorable, or mid-to-late June as the end of the feeding period of gypsy moth caterpillars approaches, *E. maimaiga* begins to produce resting spores inside the dead caterpillars, which slowly disintegrate and scatter the resting spores into the environment, with most accumulating in the soil. Laboratory determinations indicate that spores buried at least 1 cm below the surface are unable to infect gypsy moth larvae (Hajek and others 1998a). These resting spores will not germinate for approximately 9 months after production (Hajek and Humber 1997). Diet of the gypsy moth larvae could also influence the development of *E. maimaiga* (Hajek and others 1995b).

Since 1989, the fungus has spread across a large portion of the generally infested area, apparently by spore movement on the wind and intentional introduction (Elkinton and others 1991, Smitley and others 1995), infecting gypsy moth throughout its range (Hajek and others 1999). Epizootics of *E. maimaiga* have occurred in New England and some Middle Atlantic States, and its distribution continues into areas more recently colonized by the gypsy moth. The fungus is so widespread in parts of Michigan and Virginia that it is difficult to determine whether the presence of the fungus at an individual location resulted from natural migration or spread from a release (inoculation) site (Reardon and Hajek 1995).

It is not clear why the fungus suddenly appeared almost 80 years after its initial introduction into the United States. Among the hypotheses offered, the most plausible may be these two: (1) a more aggressive strain of *E. maimaiga* arose through natural selection some time after its release in 1910-1911; or (2) more of the fungus was accidentally introduced (Hajek and others 1995a, Weseloh 1998b).

Numerous constraints limit the development of *E. maimaiga* for use as an insecticide (Reardon and Hajek 1993). Fungi are often short-lived in storage and relatively expensive to produce, and foliar applications of fungi are sensitive to heat, humidity, sunlight, and rainfall. Formulation and application of dried fungal preparations also present the unique challenges of their adherence to leaf surfaces and protecting them from adverse environmental conditions.

The release of *E. maimaiga* into uninfested areas on a large scale is problematic as well. Because of its natural rate of spread, it is probably not necessary to physically introduce it into new areas. Intentional introduction of the fungus by moving soil or other inoculation into the soil would require registration and labeling of a product for this purpose with the Environmental Protection Agency (Podgwaite, John, Microbiologist, USDA Forest Service [Conversation with Joseph L. Cook]. 28 July 2004). Though *E. maimaiga* is a virulent pathogen of the gypsy moth, known to cause extensive epizootics in Japan (Shimazu and Soper 1986), it poses no known health risks to humans or pets.

E. maimaiga may eventually contribute to the long-term control of the gypsy moth. However, studies have only begun to identify the information about host-pathogen interactions that are vital to developing the fungus for effective biological control of the gypsy moth. Computer models can assist in management decisions by predicting short-term gypsy moth-fungus interactions and the effectiveness of the fungus (Weseloh 2003b).

Parasitoids.

Parasitoids live in or on another organism and benefit from the relationship, at a cost to the host, which often dies *(Figure A-5)*. Two approaches used to introduce parasitoids into the gypsy moth population in North America are classic biological control and augmentation. The discovery, importation, release, and attempted establishment of exotic natural enemies of the gypsy moth are all part of classic biological control (Reardon 1981). Manipulation to initiate or increase effective biological control through established parasites is termed augmentation (Blumenthal and others 1981).

Parasitoids, in conjunction with other natural enemies (predators and pathogens), help regulate populations of the European strain of the gypsy moth by reducing their numbers. Most researchers do not believe that they play a major role in regulating gypsy moth populations (Elkinton and Liebhold 1990).

The rate of parasitism by a particular parasitoid species varies from site-to-site and from year-to-year, depending on such factors as the number of gypsy moth caterpillars, the number of alternative hosts, and the weather. Parasitoids are thought to help maintain low-density populations of the European strain, but do not prevent the buildup of already increasing populations (Campbell 1974b). The tachinid flies, *Compsilura concinnata* (Meigen) and *Parasetigena silvestris* (Robineau-Devoidy), may play a role in suppressing incipient outbreak populations, but such population declines may go unnoticed (Elkinton and Liebhold 1990).

The State of Massachusetts and the (then) Federal Bureau of Entomology initiated foreign exploration for gypsy moth parasitoids in 1904, and the effort continues today by the USDA. Over 250,000 parasitoids of more than 85 species have been sent to the United States from collection areas around the world. Ten of these imported species were released and became established in the United States (Elkinton

Figure A-5. A parasitic wasp lays eggs on gypsy moth pupal case; eggs hatch into wasp larvae, which feed on and kill the host.

and Liebhold 1990). Additionally, several parasitoids native to the United States have become opportunistic parasitoids of the gypsy moth.

The principal egg parasitoids in North America are *Ooencyrtus kuvanae* (Howard) (Hymenoptera: Encyrtidae) and, to a much lesser degree, *Anasatus disparis* (Ruschka [Hymenoptera; Eupelmidae]). *O. kuvanae* typically attacks 10 to 40 percent of the eggs in an egg mass (Brown 1984). The rate of parasitism is greater in the smaller egg masses typical of high-density declining gypsy moth populations (Bellinger and others 1988, Brown and Cameron 1979).

Cotesia (*Apanteles*) *melanoscelus* (Ratzeburg) is a small braconid wasp that parasitizes early instar gypsy moth caterpillars, and has two generations per year. Hyperparasitoids, which prey on other parasitoids, severely reduce the numbers of *C. melanoscelus* that overwinter (Weseloh 1983). Also limiting the wasps' effectiveness is the poor synchronization of the parasitoid's second generation with its host (Weseloh 1976). Higher parasitism rates, however, reportedly occur when early gypsy moth instars are prolonged, as when they ingest sublethal doses of *B.t.k.* (Weseloh and Andreadis 1982).

Parasetigena silvestris (Diptera: Tachinidae) is a tachinid fly that lays an egg on the outer skin of the gypsy moth caterpillar, and has a single generation per year. Most active during daylight, the fly often causes more mortality than any other parasitoid. Peak parasitism tends to occur after gypsy moth populations decline from high densities (Elkinton and Liebhold 1990). In Europe, parasitism by *P. silvestris* sometimes exceeds 95 percent (Bogenschutz and others 1989).

The tachinid fly, *Blepharipa pratensis* (Meigen) (Diptera: Tachinidae), is a major source of mortality in intermediate-density gypsy moth populations (Ticehurst and others 1978) *(Figure A-6)*. It lays small eggs on foliage being fed upon by gypsy moth caterpillars. The eggs hatch after being ingested by caterpillars.

Figure A-6. Tachinid flies will parasitize gypsy moth caterpillars. (Mongolia).

Brachymeria intermedia (Nees) (Hymenoptera: Chalcididae) is a small wasp that attacks gypsy moth pupae and other hosts. Introduced in 1908 but not recovered until 1942, it was abundant by 1971 (Doane 1971). The parasitoid was observed causing high mortality of gypsy moths in Pennsylvania (Ticehurst and others 1978) and on Cape Cod (Elkinton and others 1989). *B. intermedia* tends to be scarce in low-density gypsy moth populations (Elkinton and Liebhold 1990).

Lastly, the tachinid fly *Comsilura concinnata* (Diptera: Tachinidae) has many hosts and several generations per year; it can remain abundant when gypsy moth populations are low. This fly often causes higher mortality than other parasitoids in low-density gypsy moth populations (Elkinton and Liebhold 1990).

Augmentation of these established parasitoids has not proven to be an effective means to control gypsy moth populations (Blumenthal and others 1981). Classic biological control efforts continue to be an important avenue for study, and the search for and importation of gypsy-moth-specific natural enemies from Europe and Asia remains promising.

Predators.

Many species of animals eat the gypsy moth as well as other forest-defoliating insects. Some predators feed on only one life stage of the gypsy moth, while others consume two or more life stages (Smith 1985). Predation can help maintain sparse, stable gypsy moth populations indefinitely, though periods of low predatory pressure do not necessarily lead to an outbreak. Once an outbreak starts, as well as during subsequent outbreak decline, predation has no significant effect on population densities (Smith and Lautenschlager 1981).

The gypsy moth predator community is complex and includes approximately 50 species of birds, 20 species of mammals, some amphibians, reptiles, fish, insects, and spiders. Only a few of these predators are known to affect gypsy moth population dynamics (Elkinton and Liebhold 1990, Smith and Lautenschlager 1981). The predators are all opportunistic feeders, meaning that their taste for the gypsy moth depends upon the scarcity of preferred food. Robins, for example, may eat gypsy moth caterpillars when earthworms become scarce.

Bess and others (1947) first suggested that predation by small mammals is important to gypsy moth population dynamics in North America. Vertebrate predators,

especially the white-footed mouse (*Peromyscus leucopus*) *(Figure A-7)*, are major sources of late-larval and pupal mortality in low-density gypsy moth populations (Campbell and Sloan 1977b, c, Campbell and others 1977), but not at higher gypsy moth densities (Campbell and others 1975, 1977). Small mammals help to maintain low-density gypsy moth populations (Elkinton and Liebhold 1990).

The earliest study of predation by birds, conducted by Forbush and Fernald in 1896, listed 38 bird species seen eating one or more life stages of the gypsy moth. Studies of bird predation tend to show that gypsy moth is not a major food item of most species (Cooper 1988). In feeding preference studies birds favored hairless caterpillars over gypsy moth caterpillars (Whelan and others 1989). Predation by birds is frequently cited in European literature as an important influence on gypsy moth population dynamics, but few studies exist to support that claim (Elkinton and Liebhold 1990).

The impact of invertebrate predators, such as ground beetles and ants, on gypsy moth pupae is less than that of vertebrates (Campbell and Sloan 1976, Elkinton and others 1989). Most predation by invertebrates occurs in leaf litter; little predation occurs in the tree canopy (Weseloh 1988). Adult and immature stages of *Calosoma sycophanta* (L.), a large, predaceous ground beetle introduced into North America from Europe, feed on gypsy moth caterpillars and pupae *(Figure A-8)*. *C. sycophanta* populations increase in response to high-density gypsy moth populations and tend to lag 1 to 3 years behind the onset of gypsy moth outbreaks (Weseloh 1985a, Smith and Lautenschlager 1978). The impact of *C. sycophanta* on low-density gypsy moth populations is thought to be minor (Weseloh 1985b, Smith and Lautenschlager 1978). Gypsy moth hairs defend the moth from spiders (Bardwell and Averill 1996).

Figure A-7. White-footed mice feed on gypsy moth larvae.

Figure A-8. The Calosoma beetle is a gypsy moth predator introduced from Europe.

Predators can be encouraged by maintaining habitat diversity. People unknowingly destroy good habitat for predators by removing brush in an effort to "clean up" yards and woodlots. Such cleanup efforts significantly decrease the survival of small mammals and increase the survival of gypsy moths. For example, leaving dead "snag" trees increases populations of cavity nesting birds such as woodpeckers, which eat gypsy moths. Placing nesting boxes to supplement snags may also encourage cavity-nesting birds. Leaving piles of brush might encourage populations of small mammals, such as mice and shrews, which eat gypsy moths. Forest type may also affect predation (Liebhold and others 1998). Forest thinning does not affect predation, but it was found that invertebrates are the

main predators on larvae, and small mammals the major predators on pupae (Grushecky and others 1998). As vertebrate densities increase, invertebrate predation may decrease (Cook and others 1995, Hastings and others 2002).

Nematodes.

Nematode results against defoliators such as the gypsy moth are inconsistent (Gaugler 1981, Kaya and Reardon 1982, Kaya and others 1981). Depending on the species, nematodes may actively search out their hosts and enter their body openings. In one study, two species of commercially available nematodes, *Steinernema carpocapsae* (Weiser) and *S. feltiae* (Filipjev), were applied to cloth-lined burlap and plastic bands around the tree boles to infect resting gypsy moth caterpillars. The results were highly variable between trees, primarily due to the nematode's need for a humid environment (Reardon and others 1986). Because nematodes may have potential for use against the gypsy moth, research continues.

Microsporidia.

The gypsy moth was introduced into North America without the normal complement of natural enemies that help to regulate populations in Europe. There are many groups of entomopathogens (organisms that infect insects), viruses, fungi, and protozoans found in gypsy moth populations in Europe (especially during outbreak years) that are not found in this country.

Microsporidia (protozoa) are a diverse group of obligate intracellular parasites that use most animals (including insects) and humans as hosts and are relatively host specific. According to Maddox and others (1999), six species of microsporidia described from gypsy moth populations in Europe and several isolates that have not been described or identified are recorded in the literature; microsporidia have never been reported from gypsy moth populations in North America.

The significance of microsporidian pathogens as mortality agents of gypsy moths is frequently overlooked. Among the pathogens commonly found in European gypsy moth populations, microsporidia are prevalent during the gradation period prior to outbreaks and then persist at low levels among gypsy moth populations in the years between outbreaks. Different microsporidian species that infect the gypsy moth target various tissues within their host, including the silk glands, midgut and associated muscle tissue, body fat, nerve tissue, and reproductive organs. Several authors from Europe report that microsporidia caused over 80 percent mortality of late-stage gypsy moth larvae in the Balkans and Ukraine, and caused high mortality in overwintering egg masses.

Because this strong evidence suggests that microsporidia are significant mortality factors in the dynamics of gypsy moth populations in central Europe, the USDA Forest Service initiated a foreign exploration program in 1993 to search for microsporidia in gypsy moth populations in several European countries. The program compares these isolates with previously described species and evaluates isolates that might be candidates for introduction as classical biological control agents, to enhance the natural control of this pest in North America.

Significant progress has been made in accumulating basic knowledge of the biology and life history of select isolates, which is necessary to resolve safety and regulatory issues of concern prior to consideration for possible introduction into the United States (McManus and Solter 2003). An extensive series of laboratory studies assesses the host specificity of select isolates against 49 species of non-target Lepidoptera known to occur in U.S. oak forests. Multi-year studies in Bulgaria and Slovakia evaluated the susceptibility of non-target forest Lepidoptera found with the gypsy moth in those countries. The development of molecular techniques aids in clarifying the taxonomy of European isolates and in fingerprinting individual isolates.

Preliminary discussions are ongoing with the EPA and APHIS to review the biological and ecological data accumulated on these gypsy moth pathogens during the past 10 years and to assess the feasibility of introducing them as classical biological control agents in small controlled experiments.

A.4 Miscellaneous Means of Gypsy Moth Management.

Removing and Destroying Egg Masses.

One of the first gypsy moth treatments involved removing and destroying egg masses. Broad application of this technique to control the gypsy moth reached its zenith in the 1930s with the employment of Civilian Conservation Corps workers in New England during the fall, winter, and early spring, to seek out and destroy egg masses in towns and woodlands. The technique is labor- and time-intensive and impractical for large areas. Experience has shown that in a forested area, many more egg masses are present than are actually seen and disposed of, though the technique may be helpful in urban or suburban areas on accessible trees or ornamental plantings. Careful searching, removal, and destruction of egg masses may help reduce the potential for damage due to the gypsy moth in these situations.

Tree Trunk Bands and Barriers.

As with removal and destruction of egg masses, removal and destruction of gypsy moth caterpillars may be useful in localized urban and suburban situations where small numbers of trees are at risk. The habit of caterpillars to move down from the crown and rest in protected areas during the day can be used to collect them. Bands, commonly of burlap, are placed around the trunks of susceptible trees to serve as resting areas for caterpillars seeking shelter. During an outbreak, the bands must be checked and the larvae need to be scraped off and killed. However, caterpillars may remain in the canopy and feed night and day during an outbreak, thus reducing the effectiveness of this method. Except as a survey tool, use of this technique in a forest situation is impractical.

A variety of trunk barriers is commercially available. Shown to be the most effective are barriers that include a sticky surface (Webb and Boyd 1983). An effective sticky barrier can be fashioned by wrapping the trunk of a susceptible tree with duct tape (to protect the bark and provide a smooth surface) and applying a thin layer of Tanglefoot. Gaps between the tape and the tree surface can be filled with fabric, polyester pillow stuffing, or any other suitable material. Trunk barriers should be placed just before gypsy moth eggs hatch, usually in March or April, depending on location. Insecticides can be combined with trunk barriers. A product that combines an insecticidal latex coating and burlap trunk barriers (White and others 1997) caused significant larval mortality for 30 days after application and reduced the need for manual removal of larvae.

While properly maintained sticky barriers are extremely effective at preventing caterpillars from climbing trees, they have no effect on caterpillars already in the canopy. For this reason, the impact of the barriers is usually limited to a 20- to 30-percent reduction in caterpillar numbers in treated trees over the season (Thorpe and Ridgway 1994, Thorpe and others 1993). The expected degree of foliage protection is even more variable, but usually averages 20 to 30 percent as well. Therefore, while trunk barriers provide some benefit, they should never be relied upon as the sole method to protect foliage.

Broad-Spectrum Insecticides.

A number of insecticides other than *B.t.k.*, diflubenzuron, tebufenozide, and Gypchek are registered by the U.S. EPA for gypsy moth control. These include carbaryl, which was used in the past by USDA for gypsy moth management programs. Some insecticides are registered either for gypsy moth control or for control of pests where gypsy moth is likely to be

present; for example, in areas with susceptible shade or ornamental trees. Insecticides such as carbaryl and diazinon may be available for homeowners. All of these insecticides are excluded from this SEIS because they affect a wider range of non-target organisms than do *B.t.k.*, diflubenzuron, and tebufenozide and, therefore, are not part of the USDA program. However any of these registered insecticides may be used by private applicators outside of the USDA program.

Silviculture.

Silviculture is the practice of applying treatments to forest stands to maintain and enhance their utility for any purpose (Smith 1986). Silvicultural guidelines are designed to minimize the effects of the gypsy moth on forest stands and trees and are being evaluated for effectiveness. The guidelines recommend application of treatments to minimize gypsy moth impacts before, during, and after outbreaks (Gottschalk 1993).

The greatest number of silvicultural options for gypsy moth control are available before the insect becomes established in an area. Before outbreaks, silvicultural treatments may reduce stand susceptibility and vulnerability. Treatments might include these: increasing stand and tree vigor, removing trees most likely to die, reducing gypsy moth habitat (trees with large numbers of dead branches with rough peeling bark), reducing preferred gypsy moth food sources, improving predator and parasite habitats, regenerating stands that are close to maturity or understocked, and encouraging regeneration of nonpreferred gypsy moth food sources. Silvicultural considerations in urban and suburban areas include planting trees that are less susceptible to the gypsy moth. These silvicultural treatments should be conducted at least 2 years before gypsy moth arrives in an area to allow the remaining or newly planted trees to recover from the stress of treatment.

Silvicultural techniques vary according to the condition of the site, as in converting a stand to nonpreferred species. A thinning of healthy and vigorous sites

performed 1 year before or after a gypsy moth outbreak enhances the vigor of the residual stand (Brooks and Hall 1997). When considering regeneration of preferred gypsy moth species, stump sprouts should be thinned to one stem per stump to improve vigor and resistance.

Another silviculture technique is the use of prescribed burns. This technique is occasionally used for oak regeneration. When a prescribed burn is properly used, it does not enhance susceptibility to gypsy moth.

Once the gypsy moth becomes established, or outbreaks occur or are imminent, silvicultural options are reduced. During outbreaks, silvicultural guidelines help prioritize stands that are candidates for receiving treatments and help determine if stands can be regenerated. Performing thinning during these times may reduce the density of egg masses.

Following gypsy moth outbreaks, silvicultural treatments focus on the efficient salvage of dead trees and the regeneration of stands that suffered heavy mortality or are close to maturity.

One advantage of silvicultural treatments is that action can be taken long before the gypsy moth arrives. Years might be required to treat large areas, as other resource considerations may limit the amount of cutting in an area. These treatments are prohibited in select areas, such as designated wilderness. Silviculture techniques are not quick-fixes for protection against gypsy moth or for gypsy moth suppression, but can be useful given proper planning.

A.5 Advances in Application Technology.

Advances in aerial application technology since 1990 have dramatically improved the (pilot's) applicator's ability to control aerially applied sprays, thus reducing drift and minimizing unintended environmental consequences.

The primary advance came in the early 1990s with the introduction of Differential Global Positioning System (DGPS) navigation technology for use with aerial applicator apparatus. Prior to availability of this technology, the pilot used visual markers on the ground as guidance to direct spraying. Prior to this it was difficult to know the true location of an aircraft at any instant, and standard errors in absolute position were typically around 300 meters, with no truly accurate methodology to precisely log flight paths.

DGPS navigation systems revolutionized aerial application by providing knowledge of the aircraft's absolute position within 1m (as of 2004) and the ability to log that information at 1Hz (once per second).

Use of the technology begins on the ground, marking block corners (defining the area to be treated) using DGPS and then moving this electronic file into the cockpit DGPS or by marking the desired block into a GIS system and loading that file. Most of the modern systems allow the pilot to view either a full map of the block in the cockpit or an idealized block outline. This map can be used to mark aviation hazards, landing pads, home base and other items. Current capabilities allow the pilot to automate flow control on and off functions, enabling the system to automatically turn on the spray at the block edge.

This technology is coupled with high-speed flow control, allowing precise application based on accurate aircraft position information. As powerful as the guidance functions are, the logging capabilities allow an operational manager to see exactly where the plane flew as well as evaluate the amount of material released throughout the entire operation.

Current state-of-the-art technology facilitates the use of highly accurate meteorological data and affords the ability to log release height and even provide predictions of spray movement after leaving the aircraft. These technologies greatly improve the ability of the pilot to execute an accurate, neat application—reducing costs, drift, and unintended environmental effects (Thistle 2004).

Appendix B
Gypsy Moth
Management Program

Figure B-1. Early efforts to treat the gypsy moth followed a piecemeal approach that focused on roadsides and towns.

Appendix B Gypsy Moth Management Program

Contents

Figures

This appendix describes the activities that make up the Federal gypsy moth management program, which is conducted by agencies of the U.S. Department of Agriculture under authority of public law.

B.1 General.

The gypsy moth (*Lymantria dispar*) is a nonnative invasive species. It was intentionally imported into North America in the late 1800's by a private researcher. In 1868 or 1869, near Boston, Massachusetts, it escaped. The gypsy moth has spread steadily since that time. Various strains of the gypsy moth are defoliators of forest and shade trees on four continents (Asia, Africa, Europe, and North America). The gypsy moth can cause profound changes in forest ecosystems (Work and McCullough 2000) and, in the case of severe population outbreaks, adverse human health effects (Anderson and Furniss 1983; Tuthill and others 1984).

The United States Department of Agriculture (USDA) has played a role in gypsy moth management since 1906, when Connecticut and Massachusetts first requested aid from the Federal government. The USDA Forest Service, the USDA Cooperative State Research, Education, and Extension Service (CSREES), Agricultural Research Service (ARS), and Animal and Plant Health Inspection Service (APHIS) all play a role. APHIS maintains a quarantine of the generally infested area and enforces regulations to prevent human-assisted spread of the pest (*Figure B-2*). In collaboration with State departments of agriculture, APHIS also implements an intense program for early detection and eradication of the moth when it is found outside the quarantine area. This monitoring program includes the deployment of approximately 225,000 pheromone traps nationwide, outside the quarantine area.

A memorandum of understanding between the Forest Service and APHIS identifies the roles and responsibilities in eradicating the European strain of the gypsy moth (USDA 1989). APHIS is responsible for conducting eradication projects on non-Federal lands when infestations cover less than 640 acres (259 ha). The Forest Service conducts eradication on National Forest System lands and cooperates with other agencies in projects on other Federal lands. The Forest Service also conducts eradication projects in cooperation with States on non-Federal land, when infestations cover 640 or more contiguous acres.

The USDA Forest Service carries out activities to suppress gypsy moth populations on Federal lands within the quarantine area. The Forest Service also conducts research on gypsy moth and develops tools for forest managers and others to use to help manage the insect. Slow the spread, as the name implies, is a program implemented by the Forest Service and APHIS to reduce the natural and short range artificial rate of spread of gypsy moth populations from quarantine areas to adjacent non-infested areas.

The CSREES provides technical information to businesses and landowners for management and eradication of gypsy moth on private property. ARS conducts research and evaluations on gypsy moth and development of tools to help manage the insect. ARS also conducts research and evaluations in support of the slow-the-spread strategy.

The USDA assigned responsibilities to these agencies, defined their roles to avoid duplication, and established the following policy by Departmental Regulation (USDA 1990):

• Provide a comprehensive program of gypsy moth management activities coordinated by a designated lead agency (Forest Service)
• Prevent establishment of gypsy moth outside the quarantine area;
• Develop and implement effective gypsy moth eradication and suppression measures;
• Conduct gypsy moth detection surveys and population assessments in cooperation with the States;

Historic County Quarantine for Gypsy Moth

Year of County Quarantine
- Prior 2004
- 2004
- 2005
- 2006
- 2007

0 40 80 160 240 320 Miles

Data Source: USDA APHIS 2007
www.aphis.usda.gov

AKS 10/07
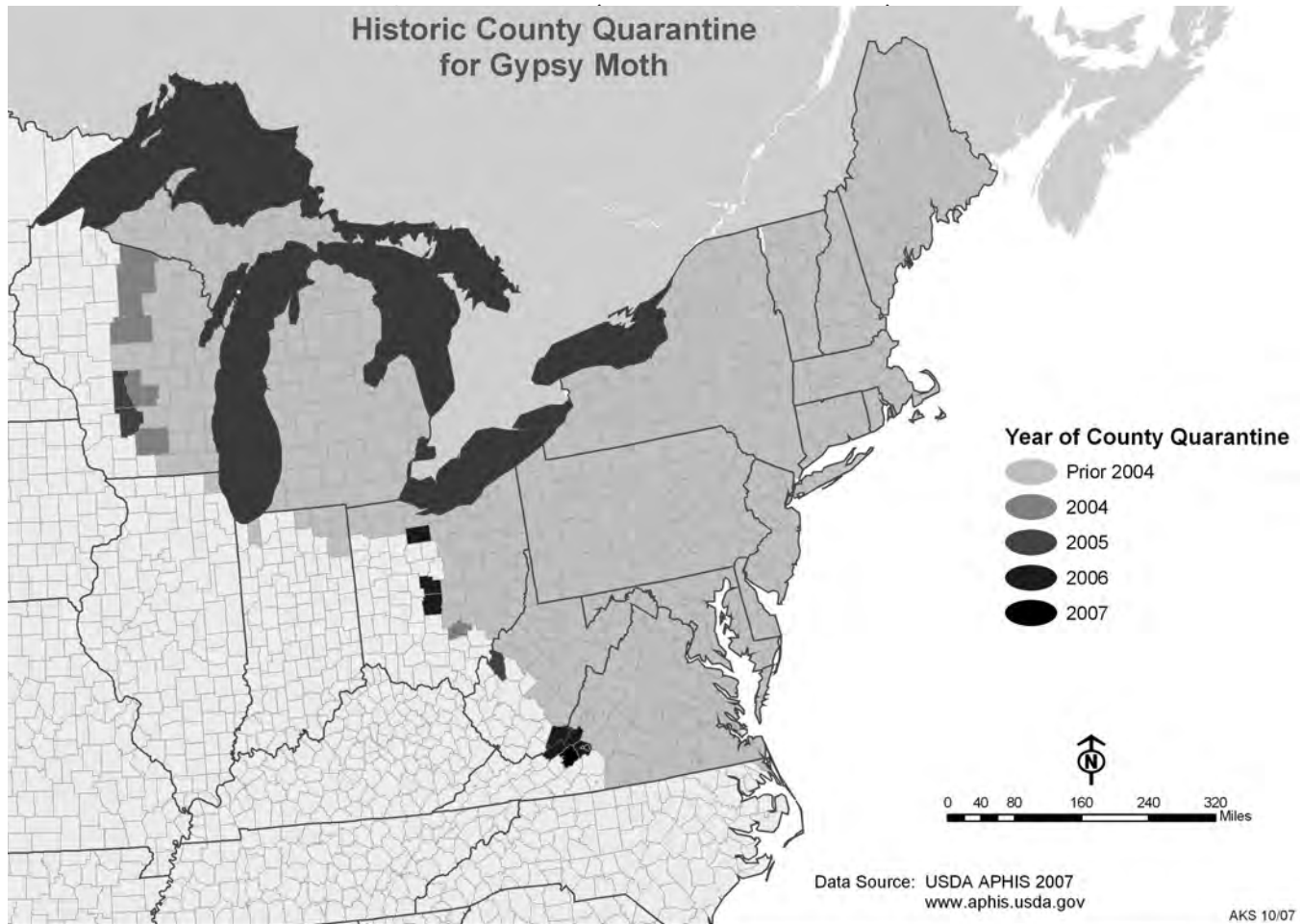
Figure B-2. As of 2006 the gypsy moth quarantine area covered all or parts of 19 States and the District of Columbia.

• Protect Federal lands and assist States in protecting non-Federal lands from gypsy moth damage;

• Plan and conduct research on the gypsy moth in partnership with the agricultural experimental stations and other cooperators, to support Federal and State gypsy moth programs;

• Prevent further introduction of the gypsy moth from abroad;

• Coordinate research planning and cooperation within USDA and other Federal and State and private agencies;

• Emphasize research deemed necessary by Federal and State cooperators from the research, extension, and action communities;

• Follow an integrated pest management approach (USDA 1993)

USDA performs its duty as defined under authority provided by several statutes:

• The Plant Protection Act (7 U.S.C. section 7701-7759)—Prevent the introduction of pests into the United States, and prevent the movement of pests across state lines.

• Cooperation with State Agencies in Administration and Enforcement of Certain Federal Laws (7 U.S.C. section 450)—Enter into cooperative agreements with States to avoid duplication of functions, facilities, and personnel and to attain closer coordination and greater effectiveness in administering Federal and State laws and regulations to control or eradicate plant pests.

• The Cooperative Forestry Assistance Act of 1978 (16 U.S.C. section 2101), as amended by the Forest

Stewardship Act of 1990 (16 U.S.C. section 2101)—Assist in controlling forest insects and diseases directly on National Forest System lands, and in cooperation with other Federal Departments and States for control of pests on other Federal land and non-Federal lands of all ownerships.

B.2 Prevention.

Port-of-Entry Activities.

APHIS is responsible for developing policies and operational guidelines to prevent the introduction of harmful, exotic agricultural quarantine organisms from entering at air, sea, and land border ports of entry. Vessels and cargo are inspected for gypsy moth contamination by the Department of Homeland Security, Customs and Border Protection.

Regulatory Activities.

The Secretary of Agriculture is authorized to quarantine States or portions of States generally infested by the gypsy moth when necessary to prevent human assisted spread of gypsy moth. Regulated articles, such as nursery stock, trees without roots, outdoor household articles, mobile homes, logs, firewood, and pulpwood, are inspected for the presence of gypsy moth life stages. Articles found to be infested are treated or cleaned of the gypsy moth life stages before movement of the articles is permitted. Public information campaigns serve to increase awareness and compliance with regulatory efforts to prevent the spread of gypsy moth.

B.3 Survey.

Surveys are conducted to monitor gypsy moth populations and to determine the extent of infestations.

Population Survey Within the Quarantine and Transition Areas.

The Forest Service monitors gypsy moth populations within the generally infested area to determine when suppression activities are warranted.   The Forest Service also tracks incipient gypsy moth populations within the transition area to guide STS activities. The Forest Service is responsible for conducting surveys within the National Forests, on other Federal lands in cooperation with Federal agencies, and on non-Federal lands in cooperation with States. Surveys are accomplished in the generally infested area primarily by visual examination for egg masses *(Figure B-3)*. Surveys are conducted in the transition area using specially designed traps baited with a manufactured version of the pheromone produced by the female gypsy moth to attract male moths.

Larval Survey.

Larval surveys may be conducted to assess development of gypsy moth caterpillars to determine the proper timing for insecticide applications. Larval surveys use sticky bands, burlap, or similar material placed around the trunks of trees to capture the larvae (USDA APHIS 1990).

Detection Survey Outside the Quarantine Area.

APHIS and the Forest Service conduct detection surveys with pheromone traps to locate new infestations and monitor treated areas.   APHIS is responsible for conducting detection surveys for gypsy moth on all lands outside the generally infested area (USDA 1989). State agencies cooperate on non-Federal lands, and Federal agencies cooperate on Federal lands. Detection surveys are conducted from late spring to late summer.

Delimiting Survey.

When adult male gypsy moths are caught, a delimiting survey using pheromone-baited traps may be used to confirm the presence of a reproducing population, the
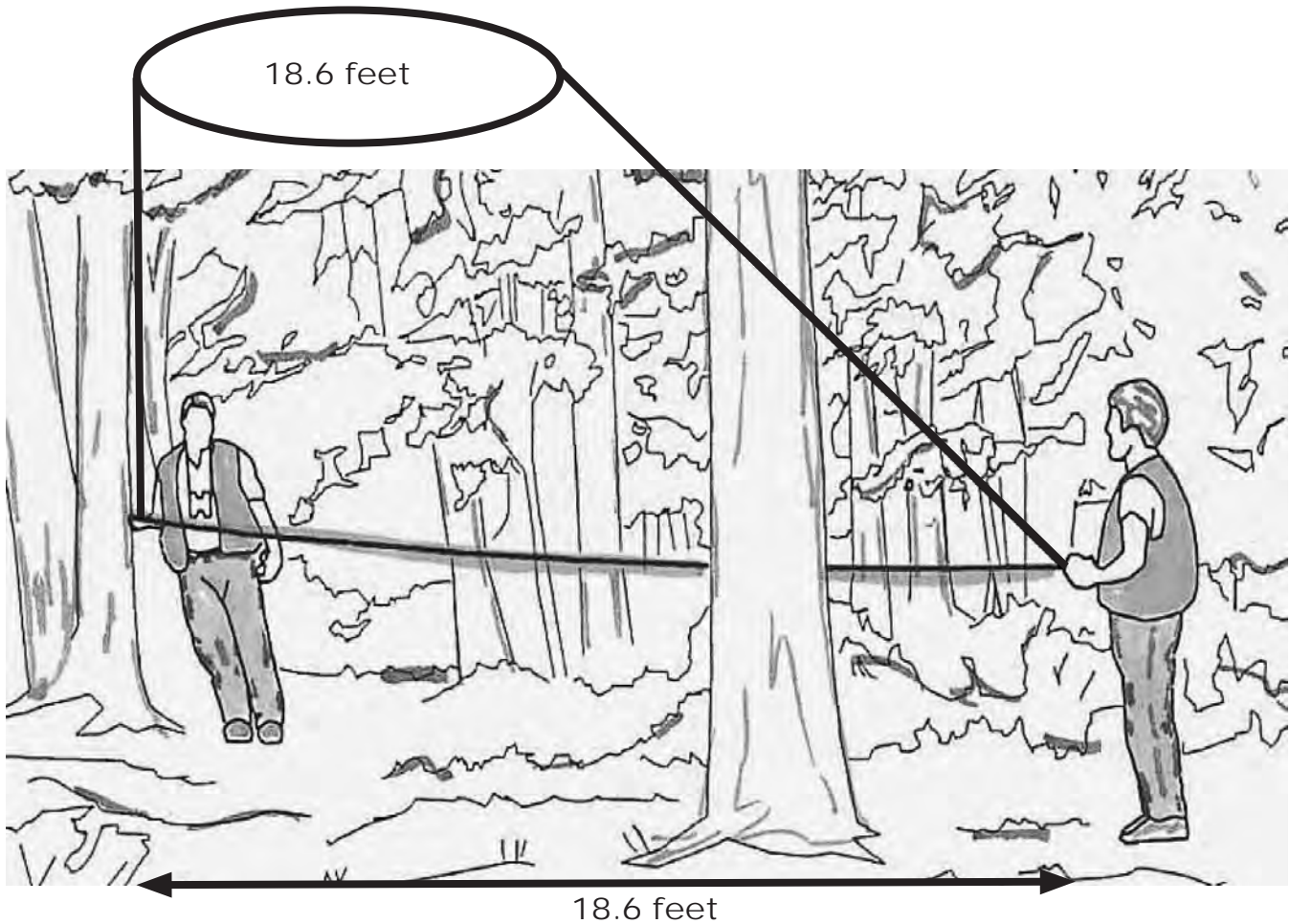
18.6 feet

18.6 feet

Figure B-3. Egg mass survey plots typically consist of 1/40-acre fixed radius plots (18.6 feet) throughout a sample area. The total sample is based on management goals of the site and distrution of host species.

approximate size of the population, and the geographic range of the infestation. The information from the delimiting survey is used to design the appropriate eradication treatment. Delimiting surveys are conducted in cooperation with the respective State governments.

B.4 Public Involvement and Notification.

The Forest Service and APHIS actively seek public participation at the local level for planned treatment projects. Before suppression, eradication, or slow-the-spread projects are carried out, public outreach is carried out and generally includes the following actions:

- Convening of public meetings facilitated by USDA;
- Identification of Federal officials who may be contacted to answer questions;
- Notification about planned treatment activities in local newspapers, and through newsletters and other media such as radio or television;
- Provision of the SEIS, environmental assessments, and related documents to agencies, groups, and individuals who are interested in the proposed action;
- Announcement of treatment dates and times to make it possible for those with concerns about insecticide application to avoid exposure.

Public meetings facilitated by USDA may include these elements:

- Presentation of the reason for the treatment project and its objective;
- Discussion of the recommended treatment and various alternatives, and their consequences;
- Soliciting of public input to identify local issues that should be addressed in the design and deployment of the project;
- Review of the details of the implementation procedure and the timing of activities.

B.5 Treatment Projects.

Any of the treatments authorized under the USDA gypsy moth management program may be conducted under any one of the strategic objectives of suppression, eradication, or slow the spread *(Figure B-4).* These strategies include planning, detection, evaluation, monitoring, and using appropriate methods to prevent establishment of new infestations, reduce damage caused by outbreaks, and slow the natural and short range spread of the gypsy moth. A project authorized under the program must be developed in compliance with Federal statutes, such as the Endangered Species Act and the National Environmental Policy Act.

Suppression.

The objective of suppression is to reduce damage caused by outbreak populations of gypsy moth in the generally infested area, thus minimizing severe defoliation of trees. Suppression does not attempt to eliminate the gypsy moth from the generally infested area, but reduces damage to ecosystems and effects on people.

Participation of State and Tribal governments or other Federal agencies in cooperative suppression projects is voluntary; private landowners may participate by coordinating with State and local agencies. In some communities, however, local nuisance ordinances or other orders may not permit private landowners to voluntary withdraw from treatments. Within the generally infested area, USDA provides assistance to
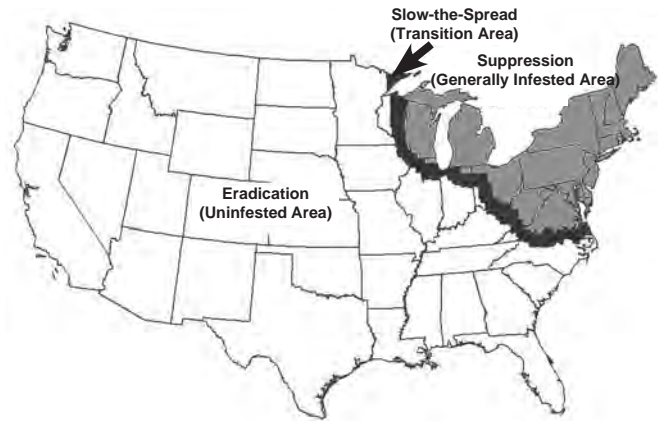
Figure B-4. Three strategies have proven succesful against the gypsy moth: suppression in the generally infested area, slow the spread in the transition area, and eradication in the uninfested area.

Federal, Tribal, and State agencies for suppression projects wherever gypsy moth outbreaks cause unacceptable levels of defoliation, by conducting projects in residential and recreational areas, forests, and special use areas, such as scenic byways and watersheds.

Site-specific environmental analyses are prepared by Federal resource managers on Federal lands, by forest supervisors on National Forests, and by Forest Service regional foresters or the Northeastern Area director on State and private lands. Gypsy moth populations are suppressed directly by the Forest Service on National Forest System lands, in cooperation with State agencies on non-Federal lands, and in cooperation with responsible officials on other Federal and Tribal lands. Proposed suppression projects must meet these criteria to be considered by the U.S. Department of Agriculture for funding (USDA Forest Service 1990b):

- Show strong potential for effective control
- Be supported by a biological evaluation that substantiates the need for the project
- Be environmentally acceptable, having met requirements of the National Environmental Policy Act
- Be supported by economic analysis, and a project work and safety plan.

Eradication.

The objective of eradication projects is to eliminate infestations detected in the uninfested area of the United States. The most common cause of isolated infestations is people moving egg masses or pupae on outdoor household articles, recreational vehicles, and boats, from the generally infested area to the uninfested area. Locations most likely to have isolated infestations in the future are wooded residential areas with high rates of relocation by people, as well as sawmills, plant nurseries, mobile home parks, and tourist attractions such as campgrounds and State and National parks.

Participation in eradication projects is governed by State law and by policies and regulations of the cooperating State agency. In some states, participation of land owners in eradication projects may be mandatory; if it is determined that State actions are inadequate, the U.S. Secretary of Agriculture can declare an emergency and conduct an eradication project.

Eradication activities may also target the Asian strain of the gypsy moth in the area generally infested by the European gypsy moth, as well as in the uninfested area. Eradication projects are conducted in cooperation with Federal and State agencies and based on the availability of Federal funds, a mutually agreed-upon plan of work, and the results of site-specific environmental analyses conducted in accordance with the National Environmental Policy Act (CEQ 1992).

Slow the Spread.

The objectives of slow the spread are to slow the natural and short-range artificial spread of the European strain of the gypsy moth from the generally infested area to uninfested areas, and to delay the adverse effects associated with infestations of new areas. Mating disruption and *B.t.k.* are used most frequently in slow-the-spread projects. Mating disruption is accomplished by the application of tiny flakes containing disparlure to confuse male gypsy moths and disrupt their normal mate-search behavior, preventing them from finding and mating with females. Slow-the-spread treatments have reduced the historic rate of spread of 13 miles per year (20.9 kilometers/year) to less than 6.25 miles per year (10.1 kilometers/year), as the gypsy moth moves into previously uninfested areas (Sharov and others 2002b).

Slow-the-spread treatments are applied in the transition area (also called the slow-the-spread action zone). When detected, gypsy moth populations are further delineated, then treated to eliminate the moths and retard their spread (*Figure B-5*) caused by "leapfrogging," which occurs when recently established populations (beyond the expanding population front) grow and coalesce, contributing to the movement of the population front (Sharov and Liebhold 1998). A more detailed description of slow the spread and how the program works can be found on page 32 of Sharov and others (2002b).

Slow the spread includes conducting intensive surveys with pheromone-baited traps to detect low-level gypsy moth populations in the transition area. Populations meeting specific criteria (based on counts of male moths, or other life stages, or both) are treated.

B.6 Monitoring and Evaluation.

The Forest Service and APHIS monitor treatment projects, with particular attention to those in environmentally sensitive areas, to ensure treatments are executed as prescribed. Environmental monitoring determines treatment effects and evaluates treated areas to assess project effectiveness.

B.7 Assistance in Planning for Forests and Trees.

The Stewardship Program, led by the Forest Service in cooperation with the States, provides technical and financial assistance for forest management planning.

STS Treatment Grid

Transition Zone populations are recently established, still at low levels, and discontinuous from one another.

2-km trap grid

STS uses grids of pheromone traps to locate and delimit isolated populations.

Isolated Colony Detected

Once these populations have been delineated, they are eradicated before they grow too large.

Delimitation Survey

STS locates and retards isolated populations in the transition zone to prevent them from growing and coalescing, thereby slowing the spread of gypsy moth.

Treatment

Figure B-5. Slow-the-spread treatments are planned in a systematic step-wise fashion.

These programs furnish an opportunity to assess potential damage from the gypsy moth and to develop contingency management plans.

The Urban and Community Forestry Program, also led by the Forest Service, encourages replacing susceptible tree species with resistant or less susceptible species (USDA Forest Service 1993). In keeping with the Forest Service's philosophy of ecosystem management, long-range tree care plans and continued inventories need to emphasize species that are less preferred by gypsy moth caterpillars. Financial and technical assistance, with the gypsy moth as a major management consideration, are available to municipalities, school districts, communities, and nonprofit organizations (but not individual landowners) for managing individual trees or groups of trees on non-Federal lands in urban environments.

B.8 Methods Development, Technology Transfer, and Research.

The Forest Service, APHIS, and Agricultural Research Service (ARS) research and identify new or improved methods of dealing with the gypsy moth. The Forest Service and APHIS also implement new technology required to support gypsy moth management activities (USDA 1990). Forest Service research develops ways to manage the gypsy moth where forests and wildlands meet urban areas, emphasizing safe and cost-effective practices that prevent populations from increasing above harmless levels and that suppress outbreaks. ARS develops the means to protect high-value trees for yards, communities, parks, and other nonforest environments and technology to support the activities of the Forest Service and APHIS. The APHIS Methods Development Center emphasizes development of gypsy moth trapping technology, pheromones, and rearing and monitoring techniques.

B.9 Information and Education.

USDA agencies participating in the Department's gypsy moth program conduct information and education activities to support their specific management responsibilities. Activities include these: developing, printing, and distributing technical publications, research reports, and briefs on the gypsy moth and gypsy moth management, preparing and distributing slide programs and videos for use in public information and education activities, developing computer software programs and geographic information systems to assist in gypsy moth management, making presentations and participating in gypsy moth workshops, and participating in public meetings and hearings.

Appendix C
Public Involvement and Issues

Figure C-1. This undated photo shows woodland defoliation caused by gypsy moths in Princeton, Massachusetts.

Appendix C Public Involvement and Issues

Contents

Figure

This appendix describes the public involvement activities that were planned and carried out for this SEIS. It states three issues that arose from the initial comments. It also summarizes comments received on the draft SEIS.

C.1 Public Involvement Activities.

Planned activities inform the public and create a process to enable comment by individuals and groups with concerns, suggestions, and ideas for shaping the content of the gypsy moth supplemental environmental impact statement (SEIS). To identify and reach the interested and affected public across the United States, the interdisciplinary team joined with public affairs and forest pest management contacts throughout the Forest Service and the Animal and Plant Health Inspection Service (APHIS) (see Chapter 5 for names of those who contributed to this document). This network also provided technical review and guidance to ensure this SEIS serves all areas of the United States. A public outreach plan was developed and implemented in June 2004. A national mailing list was compiled, and informational materials prepared about the SEIS project and the gypsy moth.

On April 29, 2004, the Forest Service and APHIS published a Notice of Intent (NOI) to Prepare a Supplement to the Final EIS for Gypsy Moth Management in the United States: a Cooperative Approach (69 Federal Register (FR) 23492-93, April 29, 2004). The public was invited to comment on the proposed supplement. Other NOIs were published on March 13, 2006 (71 FR 12674-75) and on February 7, 2007 (72 FR 5675), revising the dates for filing the draft and final SEIS.

Using a mailing list developed for the SEIS, an informational bulletin asking for comments was mailed to nearly 13,000 individuals and organizations in May 2004, including scientists, members of conservation and environmental groups, persons working in forestry

and related industries, homeowners, landowners, over 2,000 libraries, and Federal, State, and local officials. A distribution of letters to personnel within the Forest Service and APHIS solicited their input.

Team members personally met with Forest Service officials in Regions 1, 2, 3, 4, 5, 6, 8 and 9, Northeastern Area State and Private Forestry field offices, and APHIS representatives from the same areas. Team members conferred with agencies of 25 different States interested in the SEIS and gave presentations at several meetings and conferences on the gypsy moth. Attendees represented (at least) an additional 21 States, APHIS personnel from across the country, and additional Forest Service and USDA personnel with gypsy moth management and research duties from across the country. Because gypsy moth management occurs on Department of Defense lands, the team delivered a presentation, by invitation, to representatives from the Marine Corps, Air Force, Army, and Navy, at the 2004 Department of Defense Pest Management Workshop and Entomology Meeting.

Informational Bulletins.

Informational bulletins were developed and mailed throughout the development of the SEIS. Three informational bulletins have been mailed to date (March 15, 2007): the first conveyed information about the April 29, 2004, Notice of Intent to prepare an SEIS; the second bulletin provided information about the biology, host preferences, and current distribution of the moth; the third covered gypsy moth management; and the fourth covered gypsy moth research. A future informational bulletin mailing is planned on the APHIS gypsy moth program.

Periodic Press Releases.

Media releases are planned to advise of the availability of both the draft and final SEIS to generate public involvement.

C.2 Outcome and Analysis of Public Involvement Activities.

The initial comment period concluded in June 2004; all comments were acknowledged by postcard. Comments and suggestions identified from these letters and various meetings and conferences were grouped under two significant issues. Significant issues were defined as those directly or indirectly caused by implementing the proposed action. No nonsignificant issues were identified. Nonsignificant issues would have included those … (1) outside the scope of the proposed action; (2) already decided by law, regulation, Forest Plan, or other higher level decision; (3) irrelevant to the decision to be made; or (4) conjectural and not supported by scientific or factual evidence.

The Forest Service identified the following significant issues during scoping:

Issue 1— Risk to human health.

Issue 2— Risk to nontarget organisms.

Issue 1—Risk to Human Health.

The issue of human health includes the potential effects from contact with the gypsy moth and from exposure to treatments. Effects are measured by risk assessments (RAs) done for the gypsy moth and each of the treatments to include hazard identification, exposure assessment, dose-response assessment, and risk characterization. Included are the potential effects on project workers, the general public, and groups of people who may be at special or increased risk. The potential high risk group includes those who are sensitive to specific chemicals and those with multiple chemical sensitivity. Mitigation measures that can be implemented to lessen or remediate effects on human health are identified in Chapter 2.

Issue 2—Risk to Nontarget Organisms.

The issue of nontarget organisms includes potential effects due to the gypsy moth and the treatments on mammals, birds, terrestrial invertebrates, fish, and aquatic invertebrates. These effects are measured by risk assessments (RAs) done for the gypsy moth and each of the treatments, to include hazard identification, exposure assessment, dose response assessment, and risk characterization. Mitigation measures that can be implemented to remediate effects on nontarget organisms are identified in Chapter 2.

C.3 Draft SEIS Public Involvement and Comment Analysis.

THIS SECTION WILL BE COMPLETED WHEN LETTERS ARE RECEIVED FOR THE DRAFT SEIS AND MADE AVAILABLE WITH THE FINAL SEIS

Appendix D
Plant List
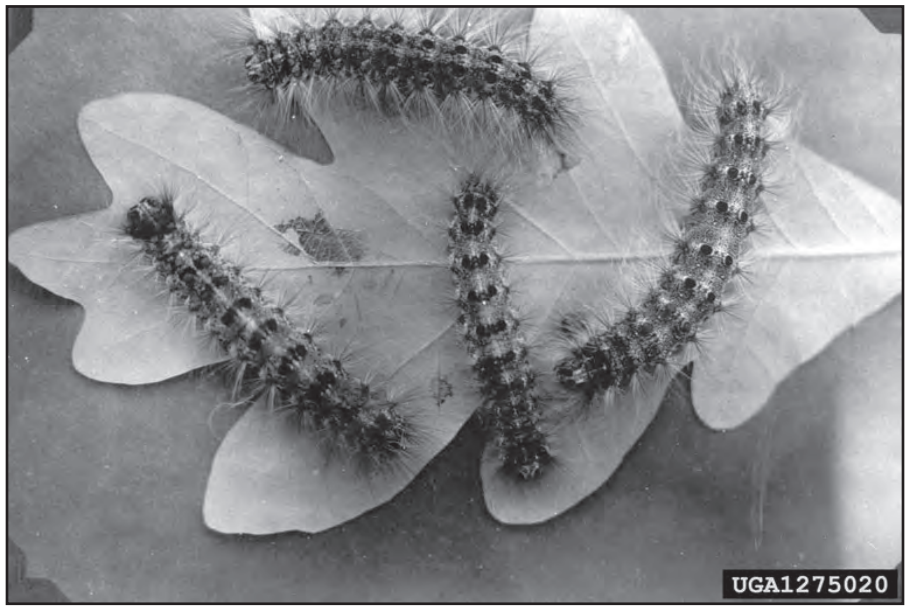
Figure D-1. White oak is one of the gypsy moth caterpillar's preferred foods.

Appendix D Plant List

Figure

This appendix lists the susceptibility of plant species to feeding by gypsy moth caterpillars (Liebhold and others 1995). The susceptibility index, based on preference and weight gain of both European and Asian strains of the gypsy moth, takes into account preference variances between strains. The index numbers provide a general ranking:

1 – Susceptible (these are plants the gypsy moth prefers to eat)

2 – Resistant (although not preferred by the gypsy moth, it will eat these plants)

3 – Immune (these species of plants are not eaten under any circumstances)

The index terms, suggested by Montgomery (1991), indicate the likelihood of plant defoliation. Plant names were selected from several sources (Dirr 1990, Little 1979, Rehder 1951, Taylor 1961, Van Dersal 1938, Viertel 1979).

Genus and species	Common name	Susceptibility index
Abelia grandiflora	glossy abelia	3
Abies amabilis	Pacific silver fir; silver fir; lovely fir; amabilis fir	2
Abies balsamea	balsam fir; Canada balsam; eastern fir	3
Abies balsamea var. *phanerolepis*	balsam fir; bracted balsam fir	3
Abies bifolia	Rocky Mountain subalpine fir	3
Abies bracteata	bristlecone fir; Santa Lucia fir; silver fir	2
Abies chinensis var. *grandiflora*	Glossy abelia	3
Abies concolor	white fir; concolor fir; silver fir	2
Abies fraseri	Fraser fir; southern balsam fir; southern fir	3
Abies grandis	grand fir; lowland white fir; lowland fir; balsam fir	2
Abies holophylla	needle fir; Manchurian fir	2
Abies lasiocarpa	subalpine fir; alpine fir; balsam fir; white balsam fir; Rocky Mountain fir	2
Abies lasiocarpa var. *arizonica*	corkbark fir	2
Abies lowiana	California white fir; white fir; Sierra white fir	2
Abies magnifica	California red fir; red fir; silvertip; golden fir	2
Abies procera	noble fir; red fir; white fir	2
Acacia baileyana	Bailey acacia; cootamundra wattel	2
Acacia farnesiana	huisache; sweet acacia; Texas huisache; cassie	2
Acacia greggii	Gregg catclaw; catclaw acacia; Texas catclaw; devilsclaw; long-flowered catclaw	2

Genus and species	Common name	Susceptibility index
Acacia longifolia	golden wattle; Sydney golden wattle	2
Acacia spp.	acacia	2
Acacia tortuosa	huisachillo; catclaw; twisted acacia; Rio Grande acacia	2
Acacia wrightii	Wright catclaw; Texas catclaw; Wright acacia	2
Acer barbatum	Florida maple; sugar maple; hammock maple	2
Acer campestre	hedge maple; English field maple	2
Acer circinatum	vine maple	2
Acer dasycarpum	silver maple; cut-leaf maple	2
Acer ginnala	amur maple	3
Acer glabrum	Rocky Mountain maple; dwarf maple; mountain maple; Sierra maple	2
Acer grandidentatum	canyon maple; bigtooth maple; sugar maple; Uvalde bigtooth maple	2
Acer japonicum	fullmoon maple	2
Acer leucoderme	chalk maple; white-bark maple	2
Acer macrophyllum	bigleaf maple; Oregon maple; broadleaf maple	2
Acer negundo	boxelder; ash-leaved maple; boxelder maple; Manitoba maple	2
Acer nigrum	black maple; black sugar maple; hard maple; rock maple	2
Acer palmatum	Japanese maple	2
Acer pensylvanicum	striped maple; moosewood	3
Acer platanoides	Norway maple	2
Acer pseudoplatanus	planetree maple; sycamore maple	2
Acer rubrum	red maple; scarlet maple; swamp maple; soft maple	2
Acer saccharinum	silver maple; soft maple; river maple; silverleaf maple	3
Acer saccharum	sugar maple; hard maple; rock maple	2
Acer spiatum	mountain maple; moose maple	3
Acer tartaricum	tartarian maple; Tartar maple	2
Achras emarginata	wild-dilly	2
Acoelorrhaphe wrightii	paurotis palm	3
Adonica merrillii	Manila palm	3
Aesculus californica	California buckeye	3
Aesculus glabra	Ohio buckeye; fetid buckeye; stinking buckeye; American horsechestnut	2

Genus and species	Common name	Susceptibility index
Aesculus hippocastanum	horsechestnut; common horsechestnut	3
Aesculus octandra	yellow buckeye; sweet buckeye; big buckeye	3
Aesculus sylvatica	painted buckeye; dwarf buckeye; Georgia buckeye	2
Ailanthus altissima	ailanthus; tree of heaven; Chinese tree-of-heaven; copaltree	2
Albizia julibrissin	silktree; mimosa; mimosa-tree; powderpuff-tree	3
Aleurites fordii	tung-oil-tree; tungtree	2
Alnus maritima	seaside alder	1
Alnus oblongifolia	Arizona alder; Mexican alder; New Mexican alder	1
Alnus rhombifolia	white alder; Sierra alder	2
Alnus rubra	red alder, Oregon alder, western alder, Pacific Coast alder	1
Alnus rugosa	speckled alder; smooth alder; tag alder; gray alder; hoary alder; hazel alder	1
Alnus serrulata	hazel alder; smooth alder; common alder; tag alder; black alder	2
Alnus sinuata	Sitka alder; mountain alder, wavyleaf alder	2
Alnus tenuifolia	mountain alder; thinleaf alder; river alder	1
Alvaradoa amorphoides	Mexican alvaradoa	2
Amelanchier alnifolia	western serviceberry; saskatoon serviceberry; serviceberry; juneberry; western shadbush	2
Amelanchier arborea	downy serviceberry; Allegheny serviceberry; shadblow; apple shadbush	2
Amelanchier canadensis	thicket serviceberry; oblongleaf juneberry	2
Amelanchier laevis	Allegheny serviceberry; downy serviceberry; smooth serviceberry	2
Amelanchier spp.	serviceberry	2
Amphitecna latifolia	black calabash	3
Amyris elemifera	torchwood; candlewood; sea amyris	2
Annona glabra	pond-apple; alligator-apple	2
Aralia spinosa	devils-walkingstick; Hercules-club; prickly-ash; angelica-tree	3
Arbutus arizonica	Arizona madrone; madrona; Arizona madrono	2
Arbutus menziesii	Pacific madrone; madrone; madrona	1
Arbutus texana	Texas madrone; madrona	2

Genus and species	Common name	Susceptibility index
Arbutus unedo	strawberry madrone; strawberrytree	2
Ardisia escallonioides	marlberry; marbleberry	2
Ardisia japonica	Japanese ardisia; marlberry	3
Arecastrum romanzoffianum	queen palm	3
Asimina triloba	pawpaw; common pawpaw; pawpaw apple; false-banana	2
Avicennia nitida	black-mangrove; blackwood	2
Betula alba	European white birch; white-barked canoe birch; cut-leaved birch	2
Betula alleghaniensis	yellow birch; gray birch; silver birch; swamp birch	3
Betula caerulea	blueleaf birch	1
Betula eastwoodiae	Yukon birch	1
Betula lenta	sweet birch; black birch; cherry birch	2
Betula nigra	river birch; red birch; black birch; water birch	1
Betula occidentalis	water birch; red birch; black birch; spring birch; paper birch	2
Betula papyrifera	paper birch; canoe birch; white birch; silver birch	1
Betula pendula	European birch; European white birch; cut-leaf weeping birch; blueleaf birch	1
Betula populifolia	gray birch; grey birch; white birch; wire birch; fire birch; oldfield birch	1
Betula pumila	swamp birch; bog birch	1
Betula verrucosa	European white birch	1
Bourreria ovata	Bahama strongback; Bahama strongbark; strongback	3
Broussonetia papyrifera	paper mulberry; common paper mulberry	3
Bumelia lanuginosa	gum bumelia; woolly buckthorn; chittamwood; swiftwig-gum; gum elastic; buckthorn	2
Bursera simaruba	gumbo-limbo; West-Indian-birch; gum-elemi	2
Callitris glaucophylla	white cypress-pine	3
Calocedrus decurrens	incense-cedar	3
Calycanthus floridus	common sweetshrub; Carolina allspice; hairy (Caroline) allspice	3
Calyptranthes pallens	pale lidflower; spicewood; white spicewood	2
Calyptranthes zuzygium	myrtle-of-the-river, spicewood	2
Canella winterana	canella; cinnamonbark; wild-cinnamon	2

Genus and species	Common name	Susceptibility index
Canotia holacantha	canotia; Mohave thorn; crucifixion-thorn	2
Capparis cynophallophora	Jamaica caper; capertree; Jamaica capertree	2
Caragana arborescens	peatree; peashrub; Siberian peashrub; Siberian pea tree	2
Carica papaya	papaya; pawpaw	2
Carpinus caroliniana	American hornbean	2
Carya aquatica	water hickory; bitter pecan; swamp hickory; bitter water hickory	2
Carya cordiformis	bitternut hickory; bitternut; swamp hickory; pignut; pignut hickory	2
Carya floridana	scrub hickory; Florida hickory	2
Carya glabra	pignut hickory; pignut	2
Carya illinoensis	pecan; sweet pecan	2
Carya laciniosa	shellbark hickory; big shellbark hickory; king nut hickory; big shagbark hickory	2
Carya leiodermis	pignut hickory; swamp hickory	2
Carya myristiciformis	nutmeg hickory; swamp hickory; bitter water hickory	2
Carya ovalis	red hickory; small pignut; sweet pignut	2
Carya ovata	shagbark hickory; shellbark hickory; upland hickory; scalybark hickory	3
Carya pallida	sand hickory; pignut hickory; pale hickory; pallid hickory	2
Carya spp.	hickory	2
Carya texana	black hickory; bitter pecan; Buckley hickory; pignut hickory	2
Carya tomentosa	mockernut hickory; mockernut; white hickory; whiteheart hickory	2
Caryota urens	toddy palm; white palm; fishtail palm; wine palm	3
Castanea dentata	American chestnut; chestnut	2
Castanea ozarkensis	Ozark chinkapin; Ozark chestnut	2
Castanea pumila	Allegheny chinkapin	2
Castanopsis chrysophylla	giant chinkapin; golden chinkapin; giant evergreen chinkapin	1
Casuarina equisetifolia	horsetail casuarina; beefwood; Australian pine; horsetail-tree	2
Casuarina stricta	coast beefwood	2
Catalpa bignonioides	southern catalpa; common catalpa; catawba; Indian-bean; cigartree	3

Genus and species	Common name	Susceptibility index
Catalpa speciosa	northern catalpa; hardy catalpa; western catalpa; catawba	3
Catalpa spp.	catalpa; hardy catalpa	3
Ceanothus arboreus	feltleaf ceanothus; island myrtle; Catalina ceanothus	3
Ceanothus integerrimus	deer brush	3
Ceanothus maritimus	ceanotus	2
Ceanothus spp.	ceanothus	3
Ceanothus thysiflorus	blueblossom; blue-myrtle; blue-brush; blueblossom ceanothus	3
Cedrus atlantica	atlas cedar	2
Cedrus deodara	deodar cedar	2
Cedrus libani	Cedar of Lebanon	2
Celtis laevigata	sugarberry; southern hackberry; Mississippi hackberry; Texas sugarberry	3
Celtis occidentalis	hackberry; northern hackberry; sugarberry; nettletree	3
Celtis tenuifolia	Georgia hackberry; dwarf hackberry; upland hackberry	3
Cephalanthus occidentalis	buttonbush; buttonball bush; honey-balls; globeflowers	2
Cercidium floridum	blue paloverde; Texas paloverde; paloverde	2
Cercidium microphyllum	yellow paloverde; littleleaf hornbeam; foothill paloverde; littleleaf paloverde	2
Cercis canadensis	eastern redbud; redbud; Judas tree	3
Cercis occidentalis	California redbud; western redbud; Arizona redbud	3
Cercocarpus betuloides	birchleaf cercocarpus; birchleaf mountain-mahogany; alderleaf cercocarpus	2
Cercocarpus breviflorus	hairy cercocarpus; Wright mountain-mahogany; hairy mountain-mahogany	2
Cercocarpus intricatus	little leaf mountain-mahogany	2
Cercocarpus ledifolius	curlleaf cercocarpus; mountain-mahogany; curlleaf mountain-mahogany	2
Cercocarpus montanus	alderleaf cercocarpus; alderleaf mountain-mahogany; mountain-mahogany; true mountain-mahogany	2
Cereus giganteus	saguaro; giant cactus; pitahaya	2
Chamaecyparis lawsoniana	Port-Orford-cedar; Port-Orford white-cedar; Oregon-cedar; Lawson cypress	3
Chamaecyparis nootkatensis	Alaska-cedar; Nootka cypress; Alaska yellow-cedar; Sitka cypress	3

Genus and species	Common name	Susceptibility index
Chamaecyparis thyoides	Atlantic white-cedar; Atlantic cedar; white-cedar; southern white-cedar	3
Chilopsis linearis	desert-willow; desert catalpa	3
Chionanthus virginicus	fringtree; fringe tree; old-mans-beard	2
Chrysobalus icaco	cocoplum	2
Chrysophyllum oliviforme	satinleaf	2
Cinnamonum camphora	camphor-tree	1
Citharexylum fruticosum	fiddlewood; Florida fiddlewood	2
Citrus aurantifolia	lime; key lime	2
Citrus limon	lemon	3
Citrus sinensis	orange; navel orange; sweet orange	2
Cladrastis lutea	yellow-wood	2
Clethra alnifolia	sweet pepperbush; summersweet clethra	3
Clethra spp.	clethra; pepperbush	3
Cliftonia monophylla	buckwheat-tree; titi; black titi	2
Coccoloba diversifolia	pigeon-plum; doveplum; tie-tongue	2
Coccoloba uvifera	seagrape; grape-tree	2
Coccothrinax argentata	Florida silverpalm; Biscayne-palm; brittle thatch; thatchpalm	3
Cocos nucifera	coconut; coconut palm	3
Colubrina reclinata	soldierwood	2
Conocarpus erectus	button-mangrove; buttonwood; silver buttonwood	2
Cordia sebestena	geiger-tree	3
Cornus alternifolia	alternate-leaf dogwood; blue cornel	3
Cornus drummondii	roughleaf dogwood	3
Cornus florida	flowering dogwood; dogwood; cornel; boxwood	2
Cornus nuttallii	Pacific dogwood; flowering dogwood; mountain dogwood	3
Cornus racemosa	gray dogwood	3
Cornus rugosa	roundleaf dogwood; roundleafed cornel	3
Cornus spp.	dogwood; cornel	3
Cornus stolonifera	red-osier dogwood; American dogwood; redstem dogwood; kinnikinnik	3
Corylus americana	American hazelnut; American filbert; wild hazelnut	1

Genus and species	Common name	Susceptibility index
Corylus avellana	European hazelnut; European filbert	1
Corylus avena		1
Corylus cornuta	beaked hazelnut; beaked filbert; western hazelnut	2
Corylus rostrata	beaked hazelnut	1
Cotinus obovatus	American smoketree; smoketree; chittamwood; yellowwood	1
Cotoneaster pyracantha	firethorn; everlasting thorn	1
Cowania mexicana	cliffrose; Stansbury cliffrose; quininebush	2
Crataegus berberifolia	barberry hawthorn; bigtree hawthorn; barberryleaf hawthorn	1
Crataegus boyntonii	Biltmore hawthorn; Boynton hawthorn	1
Crataegus brachycantha	blueberry hawthorn; blue haw; pommette blue	1
Crataegus coccinea	scarlet hawthorn; scarlet haw	1
Crataegus crus-galli	cockspur hawthorn; hog-apple; cockspur-thorn; Newcastle thorn	1
Crataegus douglasii	black hawthorn; Douglas hawthorn; river hawthorn	1
Crataegus induta	downy hawthorn; turkey hawthorn	1
Crataegus intricata	Biltmore hawthorne	1
Crataegus marshallii	parsley hawthorn; parsley-leaf hawthorn	1
Crataegus mollis	downy hawthorn	1
Crataegus monogyna	oneseed hawthorn; singleseed hawthorn; English hawthorn; European hawthorn	2
Crataegus opaca	riverflat hawthorn; English hawthorn; May hawthorn; May haw; apple haw	1
Crataegus oxyacantha	English hawthorn	1
Crataegus pedicellata	scarlet hawthorn	1
Crataegus pruinosa	frosted hawthorn; waxy-fruit thorn	1
Crataegus pyracantha	firethorn; white thorn	1
Crataegus saligna	willow hawthorn	1
Crataegus spathulata	littlehip hawthorn; small-fruit hawthorn; pasture hawthorn	1
Crataegus spp.	hawthorn	1
Cunninghamia lanceolata	China fir; blue Chinese fir	3
Cupressocyparis leylandii	Leyland cypress	3
Cupressus arizonica	Arizona cypress	3

Genus and species	Common name	Susceptibility index
Cupressus bakeri	Baker cypress; Siskiyou cypress; Modoc or MacNab cypress	3
Cupressus goveniana	Gowen cypress	3
Cupressus guadalupensis	Guadalupe cypress; Forbes' cypress; Tecate cypress	3
Cupressus macrocarpa	Monterey cypress	3
Cupressus sargentii	Sargent cypress	3
Cydonia japonica	common flowering quince; dwarf Japanese quince; Japan quince	2
Cydonia vulgaris	quince	2
Cyrilla racemiflora	swamp cyrilla; swamp ironwood; leatherwood	2
Dalea spinosa	smokethorn; smoketree; indigobush	2
Diospyros texana	Texas persimmon; black persimmon; Mexican persimmon	3
Diospyros virginiana	persimmon; common persimmon; eastern persimmon; possumwood	3
Dipholis salicifolia	willow bustic; bustic; willow-leaf bustic; cassada	2
Drypetes lateriflora	Guiana-plum	3
Elaeagnus angustifolia	Russian-olive; oleaster	3
Elaeagnus hortensis	oleaster	2
Elliottia racemosa	elliottia; southern plume	2
Enallagma latifolia	black-calabash	3
Eriobotrya japonica	loquat; loquat tree	2
Erythrina herbacea	southeastern coralbean; eastern coralbean; Cherokee-bean	2
Ethretia anacua	anaqua	3
Eucalyptus botryiodes	bastard mahogany; bangalay	2
Eucalyptus camaldulensis	longbeak eucalyptus; camal eucalyptus; redgum	2
Eucalyptus camphora	eucalyptus	3
Eucalyptus cinerea	silver dollar eucalyptus	1
Eucalyptus diversifolia	eucalyptus	3
Eucalyptus globulus	bluegum eucalyptus; Tasmanian bluegum; bluegum	2
Eucalyptus gunnii	cider gumtree	1
Eucalyptus leucoxylon	white ironbark	2
Eucalyptus polyanthemos	redbox eucalyptus; redbox-gum; Australian beech; silver dollar gum	2

Genus and species	Common name	Susceptibility index
Eucalyptus pulchella	white peppermint	2
Eucalyptus rudis	desert gum	2
Eucalyptus sideroxylon	red ironbark	2
Eucalyptus spp.	eucalyptus; gum-tree	2
Eucalyptus tereticornis	horncap eucalyptus	2
Euonymus atropurpureus	eastern burningbush; burningbush; eastern wahoo; strawberry-bush	2
Euonymus europaeus	European spindletree; European enonymus	2
Euonymus japonicus	Japanese euonymus; evergreen euonymus	2
Euonymus occidentalis	western burningbush; wahoo; western wahoo	2
Euonymus verrucosa	spindle tree	2
Exostema caribaeum	princewood; Caribbean princewood	2
Exothea paniculata	inkwood; butterbough	2
Fagus grandifolia	American beech; beech	2
Fagus sylvatica	European beech	2
Fatsia japonica	Japanese fatsia; Japanese aralia	3
Ficus aurea	Florida strangler fig; golden fig; strangler fig; wild fig	2
Ficus benjamina	Java fig; Java willow; Benjamin fig	3
Ficus carica	fig; common fig	2
Ficus elastica	India-rubber fig; rubber plant; India rubber tree	2
Ficus lyrata	fiddle-leaf fig	2
Firmiana platanifolia	Chinese parasoltree	2
Forestiera acuminata	swamp-privet; forestiera; common adelia; whitewood	3
Fraxinus americana	white ash; Biltmore ash; Biltmore white ash	3
Fraxinus anomala	singleleaf ash; dwarf ash	3
Fraxinus caroliniana	Carolina ash; water ash; Florida ash; pop ash; swamp ash	3
Fraxinus cuspidata	fragrant ash; flowering ash	3
Fraxinus excelsior	European ash	2
Fraxinus greggii	Gregg ash; littleleaf ash; dogleg ash	3
Fraxinus latifolia	Oregon ash	3
Fraxinus nigra	black ash; swamp ash; basket ash; brown ash; hoop ash; water ash	3
Fraxinus pennsylvanica	green ash; red ash; Darlington ash; white ash; swamp ash; water ash	3

Genus and species	Common name	Susceptibility index
Fraxinus profunda	pumpkin ash; red ash	3
Fraxinus quadrangulata	blue ash	3
Fraxinus spp.	ash	3
Fraxinus texensis	Texas ash	3
Fraxinus velutina	velvet ash; Arizona ash; desert ash; Modesto ash; leatherleaf ash; smooth ash; Toumey ash	3
Garrya fremontii	Fremont silktassel; silk-tassel	3
Gaultheria shallon	salal; shallon	2
Ginkgo biloba	ginkgo; maidenhair tree	3
Gleditsia aquatica	waterlocust	3
Gleditsia texana	honeylocust; Texas honeylocust	3
Gleditsia triacanthos	honeylocust; sweet-locust; thorny-locust	3
Gordonia lasianthus	loblolly-bay; tan bay; gordonia; bay; holly-bay	2
Grevillea 'noellii'	grevillea	3
Grevillea robusta	silk-oak; silky oak	3
Guaiacum sanctum	roughbark lignumvitae; holywood lignumvitae; lignumvitae	2
Guettarda elliptica	elliptic-leaf velvetseed; Everglades velvetseed; velvetseed	2
Guettarda scabra	roughleaf velvetseed	2
Gyminda latifolia	falsebox; false boxwood; West Indies falsebox	2
Gymnanthes lucida	oysterwood; crabwood	3
Gymnocladus dioicus	Kentucky coffeetree; coffeetree	3
Hakae spp.		2
Halesia carolina	Carolina silverbell; silver bell; snowdrop-tree; opossum-wood	3
Hamamelis virginiana	witch-hazel; common witch-hazel; southern witch-hazel	1
Heteromeles arbutifolia	toyon; Christmas berry; California-holly; hollyberry	2
Hibiscus rosa-sinensis	Chinese hibiscus	2
Hibiscus tiliaceus	sea hibiscus; mahoe; tree hibiscus	2
Hippomane mancinella	manchineel	3
Ilex aquifolium	English holly	3
Ilex cassine	dahoon; dahoon holly; Alabama dahoon; Christmas-berry	3
Ilex coriacea	large gallberry; tall inkberry; gallberry; bay-gallbush	3
Ilex decidua	possumhaw; deciduous holly; winterberry	3
Ilex glabra	inkberry; gallberry	3

Genus and species	Common name	Susceptibility index
Ilex krugiana	tawnyberry holly; Krug holly; southern holly	3
Ilex montana	mountain winterberry; mountain holly	3
Ilex opaca	American holly; holly; white holly	3
Ilex verticillata	common winterberry; black-alder; winterberry	3
Jasminum nudiflorum	winter jasmine	3
Juglans californica	southern California walnut; California walnut; California black walnut	2
Juglans cinerea	butternut; white walnut; oilnut	2
Juglans hindsii	northern California walnut; Hinds walnut; California black walnut	2
Juglans major	Arizona walnut; Arizona black walnut	2
Juglans microcarpa	little walnut; Texas walnut; Texas black walnut; river walnut	2
Juglans nigra	black walnut; eastern black walnut; American walnut	2
Juniperus ashei	Ashe juniper; mountain-cedar; rock-cedar; post-cedar; Mexican juniper	3
Juniperus californica	California juniper	3
Juniperus coahuilensis	redberry juniper; roseberry	3
Juniperus communis	common juniper; dwarf juniper; prostrate juniper	3
Juniperus deppeana	alligator juniper; checker-bark juniper; western juniper	3
Juniperus erythrocarpa	redberry juniper; red-fruited juniper	3
Juniperus flaccida	drooping juniper; weeping juniper; Mexican drooping juniper	3
Juniperus monosperma	oneseed juniper; cherrystone juniper; West Texas juniper	3
Juniperus occidentalis	western juniper, Sierra juniper	3
Juniperus osteosperma	Utah juniper; bigberry juniper	3
Juniperus pinchotii	Pinchot juniper; redberry juniper	3
Juniperus scopulorum	Rocky Mountain juniper; Rocky Mountain cedar; redcedar; Colorado redcedar	3
Juniperus silicicola	southern redcedar; redcedar; sand-cedar; coast juniper	3
Juniperus virginiana	eastern redcedar; redcedar; red juniper; savin	3
Krugiodendron ferreum	leadwood; black-ironwood	2
Laguncularia racemosa	white-mangrove; white buttonwood; buttonwood	2
Larix decidua	European larch	1
Larix laricina	tamarack; eastern larch; American larch; Alaska larch; hackmatack	1

Genus and species	Common name	Susceptibility index
Larix lyallii	subalpine larch; alpine larch; timberline larch; tamarack	1
Larix occidentalis	western larch; hackmatack; Montana larch; mountain larch	1
Leitneria floridana	corkwood	2
Lindera benzoin	spicebush	3
Liriodendron tulipifera	yellow-poplar; tuliptree; tulip-poplar; white-poplar	3
Lithocarpus densiflorus	tanoak; tan oak; tanbark-oak	1
Lyonia ferruginea	tree lyonia; staggerbush; titi; rusty lyonia	2
Lyonothamnus floribundus	Lyontree; Catalina-ironwood; lyonothamnus; Santa-Cruz-ironwood	2
Lysiloma bahamensis	Bahama lysiloma	2
Maclura pomifera	Osage-orange; bodark; bodock; bowwood; hedge-apple; horse-apple	3
Magnolia acuminata	cucumbertree; cucumber magnolia; mountain magnolia	3
Magnolia ashei	Ashe magnolia; sandhill magnolia	3
Magnolia fraseri	Fraser magnolia; mountain magnolia; earleaf cucumbertree	3
Magnolia grandiflora	southern magnolia; evergreen magnolia; bull-bay; big-laurel	3
Magnolia macrophylla	bigleaf magnolia; umbrella-tree; large-leaf cucumbertree	3
Magnolia pyramidata	pyramid magnolia; southern cucumbertree; mountain magnolia	3
Magnolia soulangeana	saucer magnolia; rustica rubra	3
Magnolia tripetala	umbrella magnolia; umbrella-tree; elkwood	3
Magnolia virginiana	sweetbay; swampbay; southern sweetbay; laurel magnolia	3
Malus angustifolia	southern crab apple; narrowleaf crab apple; wild crab apple	1
Malus coronaria	sweet crab apple; American crab apple; wild crab	1
Malus diversifolia	Oregon crab apple; Pacific crab apple; western crab apple; wild crab apple	1
Malus glabrata	sweet crab apple; Biltmore crab apple; wild crab	1
Malus ioensis	prairie crab apple; wild crab apple; Iowa crab	1
Malus spp.	apple	1
Melaleuca decussata	lilac melaleuca	1
Melaleuca quinquenervia	cajeput-tree; punktree; bottlebrush	2
Melia azedarach	chinaberry; umbrella chinaberry; chinatree; pride-of-India	2
Mespilus germanica	medlar; showy mespilus; European medlar	2

Genus and species	Common name	Susceptibility index
Metasequoia glyptostroboides	dawn redwood	2
Metopium toxiferum	Florida poisontree; poisonwood; West Indies poisontree	1
Morus alba	white mulberry; silkworm mulberry; weeping mulberry	3
Morus alba var. t*atarica*	Russian mulberry	3
Morus nigra	black mulberry	3
Morus rubra	red mulberry; moral	3
Morus tartarica	Tartarian mulberry	2
Mustichodendro foetidissimum	false mastic	2
Myrica californica	Pacific bayberry; California bayberry; Pacific waxmyrtle; western waxmyrtle; California waxmyrtle	2
Myrica cerifera	southern bayberry; southern waxmyrtle; bayberry; candleberry	2
Nyssa aquatica	water tupelo; tupelo-gum; cotton-gum; sourgum	3
Nyssa ogeche	Ogeechee tupelo; sour tupelo-gum; Ogeechee-lime; sour tupelo	3
Nyssa sylvatica	black tupelo; blackgum; sourgum; pepperidge; tupelo	3
Nyssa sylvatica var. *biflora*	swamp tupelo; blackgum; swamp blackgum	3
Olea europaea	olive; common olive	3
Olneya tesota	tesota; desert ironwood; Arizona-ironwood	2
Osmanthus americana	devilwood; wild-olive	3
Ostrya knowltonii	Knowlton hophornbeam; western hophornbeam; wolf hophornbeam	2
Ostrya virginiana	eastern hophornbeam; hophornbeam; American hophornbeam; hornbeam; leverwood	1
Oxydendrum arboreum	sourwood; sorrel-tree; lily-of-the-valley-tree	2
Parkinsonia aculeate	Jerusalem-thorn; horsebean; Mexican paloverde	2
Paulownia tomentosa	royal paulownia; empress-tree; princess-tree; paulownia	3
Paurotis wrightii	paurotis-palm; paurotis	3
Persea americana	avocado; zutano avocado; alligator-pear	2
Persea borbonia	redbay; shorebay	2
Photinia arbutifolia	toyon; Christmas berry	2

Genus and species	Common name	Susceptibility index
Photinia glabra	Japanese photinia	2
Photinia serrulata	Chinese photinia: Chinese medlar	2
Photinia spp.	toyon; photinia	3
Picea abies	Norway spruce	2
Picea breweriana	Brewer spruce; weeping spruce	2
Picea engelmannii	Engelmann spruce; Columbian spruce; mountain spruce; silver spruce; white spruce	2
Picea glauca	white spruce; skunk spruce; Canadian spruce; cat spruce	2
Picea mariana	black spruce; bog spruce; swamp spruce; shortleaf black spruce	2
Picea polita	tigertail spruce	2
Picea pungens	blue spruce; Colorado blue spruce; Colorado spruce; silver spruce	2
Picea rubens	red spruce; yellow spruce; West Virginia spruce; eastern spruce	2
Picea sitchensis	Sitka spruce; coast spruce; tideland spruce; yellow spruce	2
Picea spp.	spruce	2
Picramnia pentandra	bitterbush; Florida bitterbush	2
Pinckneya pubens	pinckneya; fevertree; Georgia-bark; fever-bark	2
Pinus albicaulis	whitebark pine; scrub pine; white pine	2
Pinus aristata	bristlecone pine; hickory pine; foxtail pine	2
Pinus attenuata	knobcone pine	2
Pinus balfouriana	foxtail pine	2
Pinus banksiana	jack pine; scrub pine; gray pine; black pine; Banksian pine	2
Pinus cembroides	Mexican pinyon; nut pine; Mexican stone pine	2
Pinus clausa	sand pine; scrub pine; spruce pine	2
Pinus contorta	lodgepole pine; shore pine; beach pine	2
Pinus coulteri	Coulter pine; bigcone pine; pitch pine	2
Pinus discolor	border pinyon	2
Pinus echinata	shortleaf pine; shortleaf yellow pine; yellow pine	2
Pinus edulis	pinyon; two-leaf pinyon; two-needle pinyon	2
Pinus elliottii	slash pine; yellow slash pine; swamp pine; pitch pine	2
Pinus engelmannii	Apache pine; Arizona longleaf pine	2
Pinus flexilis	limber pine; white pine; Rocky Mountain white pine	2

Genus and species	Common name	Susceptibility index
Pinus glabra	spruce pine; cedar pine; Walter pine; bottom white pine	2
Pinus halepensis	Aleppo pine	2
Pinus jeffreyi	Jeffrey pine; western yellow pine; bull pine; black pine; ponderosa pine	2
Pinus lambertiana	sugar pine; California sugar pine	2
Pinus leiophylla var. *chihuahuana*	Chihuahua pine; yellow pine	2
Pinus longaeva	intermountain bristlecone pine	2
Pinus monticola	western white pine; mountain white pine; Idaho white pine; silver pine	2
Pinus mugo	mugo pine; mountain pine; Swiss mountain pine	2
Pinus muricata	bishop pine; prickle-cone pine; Santa Cruz Island pine	2
Pinus nigra	Austrian pine; European black pine	2
Pinus palustris	longleaf pine; swamp pine; longleaf yellow pine; southern yellow pine	3
Pinus pinea	Italian stone pine	3
Pinus ponderosa	ponderosa pine; western yellow pine; yellow pine	2
Pinus ponderosa var. *arizonica*	Arizona pine; Arizona ponderosa pine; yellow pine	2
Pinus pungens	Table Mountain pine; mountain pine; hickory pine	2
Pinus quadrifolia	Parry pinyon; four-needle pinyon; nut pine	2
Pinus radiata	Monterey pine; insignis pine	2
Pinus resinosa	red pine; Norway pine	2
Pinus rigida	pitch pine	3
Pinus sabiniana	Digger pine; bull pine; gray pine	2
Pinus serotina	pond pine; marsh pine; pocosin pine	2
Pinus spp.	Pine	2
Pinus strobiformis	southwestern white pine; Mexican white pine; border white pine	2
Pinus strobus	eastern white pine; northern white pine; white pine	2
Pinus sylvestris	Scotch pine; Scots pine	2
Pinus taeda	loblolly pine; oldfield pine; shortleaf pine	2
Pinus thunbergiana	Japanese black pine	3

Genus and species	Common name	Susceptibility index
Pinus torreyana	Torrey pine; Del Mar pine; Soledad pine	2
Pinus virginiana	Virginia pine; Virginia scrub pine; spruce pine; Jersey pine; scrub pine; poverty pine	2
Pinus washoensis	Washoe pine	2
Piscidia piscipula	Florida fishpoison-tree; Jamaica-dogwood; Florida fishfuddletree	2
Pistacia texana	Texas pistache; American pistachio; wild pistachio	1
Pistacia vera	pistachio	1
Planera aquatica	water-elm; planertree	2
Platanus orientalis	Oriental planetree	2
Platanus racemosa	California sycamore; western sycamore; California planetree	3
Platanus wrightii	Arizona sycamore; Arizona planetree	3
Populus alba	white poplar; silver poplar	2
Populus angustifolia	narrowleaf cottonwood; black cottonwood; mountain cottonwood; narrowleaf poplar	1
Populus balsamifera	balsam poplar; balm; balm-of-Gilead; bam; tacamahac	1
Populus deltoides	eastern cottonwood; eastern poplar; southern cottonwood	2
Populus fremontii	Fremont cottonwood; cottonwood	2
Populus grandidentata	bigtooth aspen; largetoothed aspen; aspen; poplar; popple	1
Populus heterophylla	swamp cottonwood; black cottonwood; river cottonwood	1
Populus nigra var. *italica*	Lombardy poplar	1
Populus palmeri	eastern cottonwood; eastern poplar; Palmer cottonwood	1
Populus sargentii	plains cottonwood; great plains cottonwood; sargent cottonwood	1
Populus spp.	cottonwood; poplar	1
Populus tremuloides	quaking aspen; trembling aspen; golden aspen	1
Populus trichocarpa	black cottonwood; western balsam poplar; cottonwood; balsam cottonwood	1
Populus wislizenii	Rio Grande cottonwood; valley cottonwood	1
Prosopis juliflora	honeylocust; mesquite; algaroba	2
Prosopis pubescens	screwbean mesquite; screwbean	2
Prunus alleghaniensis	Allegheny plum; sloe plum; sloe; Allegheny sloe; northern sloe	2

Genus and species	Common name	Susceptibility index
Prunus americana	American plum; wild plum; red plum; river plum; yellow plum	2
Prunus angustifolia	Chickasaw plum; sand plum	2
Prunus avium	mazzard; common sweet cherry; English cherry	2
Prunus caroliniana	Carolina laurelcherry; laurel cherry; cherry-laurel	2
Prunus domestica	garden plum; plum; Damson plum	2
Prunus emarginata	bitter cherry; quinine cherry; wild cherry	2
Prunus fremontii	desert apricot	2
Prunus glandulosa	flowering almond; dwarf flowing almond; almond cherry; wild peach	2
Prunus hortulana	Hortulan plum	2
Prunus japonica	Japanese plum	2
Prunus laurocerasus	cherry laurel; English laurel	2
Prunus lyonii	Catalina cherry	2
Prunus maritima	beach plum	2
Prunus mexicana	Mexican plum; bigtree plum; inch plum	2
Prunus munsoniana	wildgoose plum; Munson plum	2
Prunus myrtifolia	West Indies cherry; myrtle laurel cherry; laurelcherry	2
Prunus nigra	Canada plum; red plum; horse plum; wild plum	2
Prunus padus	European bird-cherry; black serviceberry	2
Prunus pensylvanica	pin cherry; wild red cherry; fire cherry; northern pin cherry; pigeon cherry; bird cherry	3
Prunus persica	peach; nectarine; heavenly white nectarine; Tilton apricot	2
Prunus pissardi	purple-leaved prune	2
Prunus pumila	sand cherry	2
Prunus serotina	black cherry; wild black cherry; rum cherry; mountain black cherry	2
Prunus spinosa	sloe; blackthorn	2
Prunus spp.	cherry; plum	2
Prunus subcordata	Klamath plum; Sierra plum; Pacific plum; western plum; wild plum	2
Prunus umbellata	flatwoods plum; black sloe; hog plum; sloe	2
Prunus virginiana	chokecherry; common chokecherry; black chokecherry; California chokecherry	2

Genus and species	Common name	Susceptibility index
Pseudophoenix sargentii	buccaneer-palm; Florida cherrypalm; Sargent cherrypalm	3
Pseudotsuga macrocarpa	bigcone Douglas-fir; bigcone-spruce; hemlock	2
Pseudotsuga menziesii	Douglas-fir; red-fir; Oregon-pine; Douglas-spruce	2
Psidium guajava	guava; common guava; guayaba	2
Ptelea trifoliata	hoptree; common hoptree; wafer-ash	2
Punica granatum	pomegranate	2
Pyracantha coccinea	scarlet firethorn; everlasting thorn; fire thorn	2
Pyrus angustifolia	narrowleaf crab apple	1
Pyrus arbutifolia	red chokecherry; red chokeberry; chokeberry	2
Pyrus communis	pear	2
Pyrus fusca	Oregon crab apple	1
Pyrus malus	wild apple; common apple	1
Quercus agrifolia	coast live oak; California live oak	1
Quercus alba	white oak; stave oak	1
Quercus arizonica	Arizona white oak; Arizona oak	1
Quercus austrina	Durand oak; Durand white oak; bluff oak	1
Quercus bicolor	swamp white oak	1
Quercus chapmanii	Chapman oak; Chapman white oak; scrub oak	1
Quercus chrysolepis	canyon live oak; California live oak; canyon oak; goldcup oak; live oak; maul oak	1
Quercus cinerea	bluejack oak	1
Quercus coccinea	scarlet oak; black oak; Spanish oak	1
Quercus douglasii	blue oak; California blue oak; iron oak; mountain white oak; mountain oak	1
Quercus durandii	Durand oak; Durand white oak; bluff oak; white oak	1
Quercus ellipsoidalis	northern pin oak; jack oak; black oak; Hill oak	1
Quercus emoryi	Emory oak; black oak; blackjack oak	1
Quercus engelmannii	Engelmann oak; evergreen white oak; mesa oak; Engelmann spruce	1
Quercus falcata	southern red oak; Spanish oak; water oak; red oak	1
Quercus gambelii	Gambel oak; Rocky Mountain white oak; Utah white oak; white oak	1

Genus and species	Common name	Susceptibility index
Quercus garryana	Oregon white oak; Oregon oak; Garry oak; post oak; white oak; Brewer oak; shin oak	1
Quercus grisea	gray oak; Arizona gray oak	1
Quercus hemisphaerica	laurel oak; Darlington oak	1
Quercus hypoleucoides	silverleaf oak; white-leaf oak	1
Quercus ilicifolia	bear oak; scrub oak	1
Quercus imbricaria	shingle oak; laurel oak	1
Quercus incana	bluejack oak; cinnamon oak; sandjack; bluejack; shin oak; turkey oak	1
Quercus kelloggii	California black oak; black oak; Kellogg oak	1
Quercus laevis	turkey oak; Catesby oak; scrub oak	1
Quercus laurifolia	laurel oak; Darlington oak; diamond-leaf oak; swamp laurel oak	1
Quercus lobata	valley oak; California white oak; valley white oak; water oak	1
Quercus lyrata	overcup oak	1
Quercus macrocarpa	bur oak; mossy cup oak; blue oak; mossy-overcup oak; scrub oak	1
Quercus margaretta	sand post oak; small post oak; dwarf post oak; post oak	1
Quercus marilandica	blackjack oak; blackjack; barren oak; black oak; jack oak	1
Quercus michauxii	swamp chestnut oak; basket oak; cow oak	1
Quercus muehlenbergii	chinkapin oak; yellow chestnut oak; chestnut oak; rock chestnut oak	1
Quercus myrtifolia	myrtle oak; scrub oak	1
Quercus nigra	water oak; possum oak; spotted oak	1
Quercus nuttallii	Nuttall oak; red oak; Red River oak; pin oak	1
Quercus oblongifolia	Mexican blue oak	1
Quercus oglethorpensis	Oglethorpe oak	1
Quercus pagoda	cherrybark oak; swam red oak; bottomland red oak	1
Quercus palustris	pin oak; swamp oak; water oak; swamp Spanish oak; Spanish oak	1
Quercus phellos	willow oak; pin oak; peach oak; swamp willow oak	1
Quercus prinus	chestnut oak; basket oak; rock chestnut oak; rock oak; tanbark oak	1
Quercus rubra	northern red oak; red oak; common red oak; gray oak; eastern red oak; mountain red oak	1

Genus and species	Common name	Susceptibility index
Quercus shumardii	Shumard oak; Shumard red oak; spotted oak; Schneck oak; Schneck red oak; southern red oak	1
Quercus spp.	oak	1
Quercus stellata	post oak; iron oak	1
Quercus suber	cork oak	1
Quercus undulata	Rocky Mountain shin oak; wavyleaf oak	1
Quercus velutina	black oak; yellow oak; quercitron oak; yellow-bark oak; smooth-bark oak	1
Quercus virginiana	live oak; Virginia live oak	1
Quercus wislizenii	interior live oak; highland live oak; Sierra live oak	1
Rapanea guianensis	Guiana rapanea	2
Reynosia septentrionalis	darling-plum; red-ironwood	2
Rhamnus caroliniana	Carolina buckthorn; Indian-cherry; yellow buckthorn; tree buckthorn; yellowwood	3
Rhamnus cathartica	European buckthorn; common buckthorn; European waythorn	3
Rhamnus frangula	glossy buckthorn; alder buckthorn	3
Rhamnus purshiana	cascara buckthorn; cascara; cascara sagrada; bearberry; chittam; coffeetree	2
Rhizophora mangle	mangrove; red mangrove	2
Rhus copallina	shining sumac; dwarf sumac; winged sumac; wing-rib sumac; flameleaf sumac	2
Rhus corallina	mountain sumac	1
Rhus cotinus	smoketree; common smoketree	2
Rhus glabra	smooth sumac; scarlet sumac; common sumac; Rocky Mountain sumac; red sumac	1
Rhus integrifolia	lemonade sumac; sourberry; lemonade-berry; mahogany sumac	2
Rhus typhina	staghorn sumac; velvet sumac	1
Ribes uva-crispa	English gooseberry	2
Robinia neomexicana	New Mexico locust; New Mexican locust; southwestern locust	3
Robinia pseudoacacia	black locust; common locust; yellow locust; white locust	3
Robinia spp.	locust	2

Genus and species	Common name	Susceptibility index
Robinia viscosa	clammy locust	3
Rosa bracteata	Macartney rose	2
Rosa eglanteria	sweetbriar; sweetbriar rose	2
Rosa setigera	prairie rose; climbing prairie rose	2
Rosa spp.	rose	1
Roystonea elata	Florida royalpalm; Cuban royalpalm; royalpalm	3
Sabal palmetto	cabbage palmetto; common palmetto; Carolina palmetto; palmetto; cabbage-palm	3
Salix alaxensis	feltleaf willow	1
Salix alba	white willow; European white willow	1
Salix alba var. *tristis*	golden weeping willow	1
Salix amygdaloides	peachleaf willow; peachleaved willow; almond willow; peach willow; southwestern peach willow	1
Salix babylonica	weeping willow; Babylon weeping willow; Napolean willow	2
Salix bonplandiana	Bonpland willow; Toumey willow; red willow; polished willow	1
Salix caroliniana	Coastal Plain willow; Ward willow; southern willow; Harbison willow	1
Salix cordata	heartleaf willow; heart-leaved willow	1
Salix discolor	pussy willow; glaucous willow; silvery pussy willow	1
Salix eriocephala	pussy willow	1
Salix fragilis	crack willow; brittle willow; snap willow	1
Salix hookerana	Hooker willow; coast willow; Yakutat willow; bigleaf willow	1
Salix interior	sandbar willow; coyote willow; acequia willow; basket willow; gray willow; sandbar willow	1
Salix laevigata	Bondpland willow; red willow; Toumey willow; polished willow	1
Salix lasiandra	Pacific willow; whiplash willow; black willow; red willow; western black willow; yellow willow	1
Salix lasiolepis	arroyo willow; white willow	1
Salix lucida	shining willow; shiny willow	1
Salix mackenzieana	Mackenzie willow	1
Salix nigra	black willow; swamp willow; Goodding willow; western black willow; Dudley willow	1
Salix pentandra	laurel willow; bay willow; bayleaf willow	2

Genus and species	Common name	Susceptibility index
Salix scouleriana	Scouler willow; fire willow; black willow; mountain willow; Nuttall willow	1
Salix spp.	willow	1
Salix taxifolia	yewleaf willow; yew willow	1
Salix viminalis	basket willow; osier; common osier; silky osier	1
Sambucus callicarpa	Pacific red elder; Pacific elder; coast red elder; redberry elder; red elderberry	2
Sambucus canadensis	American elder; common elderberry; common elder; blackberry elder	3
Sapindus drummondii	western soapberry; wild chinatree; cherioni	2
Sapindus marginatus	wingleaf soapberry; Florida soapberry	2
Sapindus saponaria	wingleaf soapberry; Florida soapberry; southern soapberry; Mexican soapberry; wild chinatree	2
Sapium sebiferum	tallowtree; Chinese tallowtree	3
Sassafras albidum	sassafras; white sassagras	2
Schinus molle	California peppertree	1
Sequoia sempervirens	redwood; coast redwood; California redwood	2
Sequoiadendron giganteum	giant sequoia; sequoia; bigtree; Sierra redwood	2
Sideroxylon foetidissimum	false-mastic; mastic; wild-mastic; wild-olive	2
Simarouba glauca	paradise-tree; bitterwood	2
Sophora affinis	Texas sophora; coralbean; pink sophora; Eves-necklace	3
Sophora japonica	Japanese pagoda-tree	3
Sophora secundiflora	mescalbean; frigolito; coralbean; Texas-mountain-laurel	2
Sorbus americana	American mountain-ash; mountain-ash; roundwood	1
Sorbus aucuparia	European mountain-ash; Rowan-tree	1
Spiraea bumalda	Bumalda spirea; spirea	3
Stewartia koreana	Korean stewartia; stewartia	3
Stewartia ovata	mountain stewartia; mountain-camellia; angel-fruit stewartia	2
Swietenia mahagoni	West Indies mahogany; mahogany	2
Symphoricarpos albus	snowberry; waxberry; common snowberry	3
Symplocos tinctoria	sweetleaf; horse-sugar; common sweetleaf; yellowwood	2
Tamarix parviflora	small-flower tamarisk	2
Taxodium distichum	baldcypress	3

Genus and species	Common name	Susceptibility index
Taxodium mucronatum	Montezuma baldcypress; Mexican cypress	3
Taxus brevifolia	Pacific yew; western yew	3
Taxus floridana	Florida yew	3
Thrinax microcarpa	key thatchpalm; silvertop palmetto; prickly thatch; brittle thatch; brittle thatch palm	3
Thrinax parviflora	Jamaica thatchpalm	3
Thuja occidentalis	northern white-cedar; white-cedar; eastern arborvitae; American arborvitae; eastern white-cedar	3
Thuja orientalis	oriental arborvitae; Chinese arborvitae	3
Thuja plicata	western redcedar; giant western arborvitae; Pacific redcedar; giant-cedar; arborvitae; canoe-cedar	3
Tilia americana	American basswood; American linden; basswood	1
Tilia caroliniana	Carolina basswood; Florida basswood; basswood; Carolina linden; Florida linden	1
Tilia cordata	littleleaf linden; small-leaved linden; small-leaved European linden	1
Tilia europaea	European linden	1
Tilia floridana	Florida basswood; Carolina basswood	1
Tilia heterophylla	white basswood; beetree; linden; beetree linden	1
Torreya californica	California torreya; California-nutmeg	3
Torreya taxifolia	Florida torreya; stinking-cedar	3
Torrubia longifolia	longleaf blolly; Brace blolly roundleaf blolly; beeftree; beefwood	2
Toxicodendron vernix	poison-sumac; poison-dogwood; poison-elder; thunderwood	1
Trema micrantha	Florida trema	2
Tsuga canadensis	eastern hemlock; Canadian hemlock; Canada hemlock; hemlock spruce; common hemlock	2
Tsuga caroliniana	Carolina hemlock	2
Tsuga heterophylla	western hemlock; Pacific hemlock; west coast hemlock	2
Tsuga mertensiana	mountain hemlock; black hemlock; alpine hemlock; hemlock spruce	2
Ulmus alata	winged elm; wahoo elm; cork elm; wahoo	2
Ulmus americana	American elm; white elm; water elm; soft elm; Florida elm	2
Ulmus campestris	English elm; European elm	2
Ulmus crassifolia	cedar elm; basket elm; red elm; southern rock elm	2

Genus and species	Common name	Susceptibility index
Ulmus glabra	Scotch elm; wych elm	2
Ulmus montana	Scotch elm	2
Ulmus parvifolia	Chinese elm; lacebark	2
Ulmus pumila	Siberian elm; Asiatic elm; dwarf Asiatic elm; Pekin elm	2
Ulmus racemosa	rock elm; cork elm	2
Ulmus rubra	slippery elm; red elm; gray elm; soft elm	3
Ulmus serotina	September elm; red elm	2
Ulmus spp.	elm	2
Ulmus thomasii	rock elm; cork elm	2
Umbellularia californica	California-laurel; California-bay; Oregon-myrtle; Pacific-myrtle; pepperwood; spice-tree	3
Vauquelinia californica	Torrey vauquelinia; Arizona-rosewood	2
Veitchia merrillii	Manila palm	3
Viburnum acerifolium	mapleleaf viburnum; dockmackie; maple-leaved arrowwood	3
Viburnum ellipticum	western blackhaw; oval-leafed virburnum	2
Viburnum lantana	wayfaringtree	2
Viburnum opulus	European cranberrybush; highbush cranberry; cranberry tree	3
Viburnum prunifolium	blackhaw; stagbush; sweethaw	2
Viburnum pubescens	downy viburnum; hairy nannyberry; downy arrowwood	2
Viburnum rhytidophyllum	leatherleaf viburnum	2
Viburnum spp.	viburnum; wayfaringtree	3
Viburnum tomentosum	doublefile viburnum	3
Washingtonia filifera	California washingtonia; California-palm; fanpalm; California fanpalm; desert-palm	3
Ximenia americana	tallowwood; hogplum	3
Zanthoxylum americanum	common prickly-ash; toothache-tree; northern prickly-ash; prickly ash	2
Zanthoxylum clava-herculis	Hercules-club; pepperbark; southern prickly-ash; toothache-tree; tingle-tongue	2
Zanthoxylum fagara	lime prickly-ash; wild-lime-tree; wild-lime	2
Zanthoxylum flavum	West Indies satinwood; yellowheart; satinwood; yellowwood	2

Appendix E
Biology, History, and Control Efforts for the Gypsy Moth

Figure E-1. Small hand sprayers were used to apply DDT in 1945 (Gill, MA).

Appendix E Biology, History, and Control Efforts for the Gypsy Moth

Contents

Figures and Tables

This appendix describes the progression of control efforts that paralleled the spread of the gypsy moth from 1869 to 2005 in the United States *(Figure E-2)*. Biological information includes coverage of its life cycle, differences between the European and Asian strains, and the four population phases and host plants.

E.1 About the Gypsy Moth.

The following information is provided to facilitate better understanding of the insect, the problems it creates, and treatments.

Life Cycle.

Producing one generation per year, the gypsy moth goes through four life stages: egg, larva, pupa, and adult moth *(Figures E-3, E-4, E-5, E-6)*.

After mating, the female gypsy moth deposits eggs in a well-defined mass, containing from a few hundred to a thousand eggs, typically in a protected area such as bark crevices, on the underside of branches, and in leaf litter. She coats the eggs with hairs from her abdomen, giving the egg mass a furry appearance and buff color.

Though the embryos within the eggs develop into caterpillars in 4 to 6 weeks, the caterpillars remain in the eggs during winter. Survival and hatching success depend on a combination of time and temperature requirements. A prolonged period of chilling and sufficient time for subsequent incubation are necessary for egg hatch the following spring (Giese and Casagrande 1981).

Figure E-2. A historic county quaratine map shows the spread of the gypsy moth from 1909 to 2007.

Figure E-3. Female gypsy moths add hairs from their abdomens to their egg masses.

Figure E-4. The gypsy moth catepillar (larva) develops pairs of distintive red and blue spots as it grows.

Figure E-5. The gypsy moth pupa lasts for about 2 weeks.

Figure E-6. The gypsy moth adult male (left) and female (right) are visibly different.

Coinciding with the appearance of spring leaves, eggs laid the previous year hatch (during April and May in the Middle Atlantic States), and caterpillars climb multiple varieties of trees, bushes, and other objects, spinning a thread of silk from which they hang freely. A phenomenon termed "ballooning," by which the wind carries them to new locations, relocates most caterpillars before they begin feeding. Caterpillars may balloon several times before they settle and begin feeding on foliage (Nichols 1980).

The small caterpillars move into the tree canopy where they feed on leaves for the next 6 to 8 weeks. Caterpillars grow from one-tenth of an inch (3 mm) to as large as 3½ inches (90 mm) by going through a

series of growth stages called instars. A molt (shedding of the outer layer) precedes each instar stage; the discarded "skins" can cause respiratory problems for some people. Male caterpillars grow through five instars, females through six; an additional instar is not uncommon (Doane and McManus 1981).

The caterpillars develop distinctive markings on their ash-colored bodies as they grow--a yellow stripe down the back, with rows of five blue spots followed by six red spots on both sides of the yellow stripe. Their excrement, called frass, can create health risks; large populations of caterpillars excrete so much frass it sounds like rain falling through the leaves. Frass in runoff water can also pollute lakes and streams, threatening fish (Sharpe 1982).

Caterpillars typically feed at night to avoid predators, though feeding may occur at any time of the day when caterpillar populations explode and competition for food increases. The feeding caterpillar is the life stage targeted in most gypsy moth treatment projects because of the potential for defoliation.

When population levels are low, caterpillars move down the tree during the day and rest in protected areas under tree bark and in crevices, returning to the tree canopy to feed at night. When populations are elevated and competition for foliage high, caterpillars remain in the tree canopy and feed night and day. After stripping the foliage of the host tree, the caterpillars descend, crawling in search of new food sources (McManus and others 1989).

Following the last instar (June and July in the Middle Atlantic States), caterpillars find any available protected spot in trees, on buildings, and even on the ground, entering their pupal stage over the next 2 days. Approximately 2 weeks later, adult moths emerge.

Male gypsy moths appear first, followed several days later by the females. The egg-laden females emit a pheromone, attracting males for mating. The female

moths then deposit their egg masses, beginning the cycle anew the following spring.

European and Asian Strains.

The European strain of the gypsy moth became established in North America from a single introduction of closely related individuals, and genetic studies have shown little variation within or between populations (Wallner 1992). In North America, the European strain is also called the North American strain.

The common reference to "the Asian strain" of the gypsy moth is actually several strains, which display considerable variability. The most notable variances are the female's flying abilities (some females of the Asian strain are strong fliers, capable of flights exceeding 18 miles [28.9 km]) and the capacity to establish in a broad range of hosts (Wallner 1992).

The European and Asian strains of the gypsy moth are similar in appearance; however, behavioral differences between them are significant, particularly the inability of the European strain female to fly (Wallner 1992). Females of the Asian strain are attracted to light and more likely to deposit their eggs near light sources, thus potentially increasing the social "nuisance" factor usually associated with the gypsy moth (Hofacker 1994).

The Asian strain feeds on some hosts that are only marginally acceptable to the European strain, increasing their potential to establish themselves and cause even more extensive defoliation than their European cousins (USDA APHIS 1992).

Other differences between the European and Asian strains are minor *(Table E-1)*. The most reliable method for distinguishing between the strains, other than the flight of the female, is genetic testing .

Prior to the first known introductions of the Asian strain in 1991, eradication actions were singularly focused against the European strain. Efforts against

Table E-1. Differences between the European and Asian strains of the gypsy moth, by life stage and cause of mortality (adapted from Wallner 1992, p. 2).

Life Stage	European Strain (North America)	Asian Strain (Siberia, Russia, Far East)
Caterpillars	First instars disperse Color uniform Main hosts: oak, birch, poplar, willow, alder Early instars feed in the canopy at night and move to resting sites during the day.	First and second instars disperse Color highly variable Main hosts: oak, larch, birch, willow Early instars feed in the canopy at night and remain on the host during the day.
Pupae	Pupates in protected spots in bark crevices, in leaf litter	Pupates on foliage
Adult Females	Flightless	Strong flier, attracted to light
Egg Masses	On tree trunks, rocks, leaf litter	On foliage, tree trunks, rocks, objects near lights
Cause of Mortality	Virus, *B.t.*, fungus, parasites, various predators	Virus, *B.t.*, fungus, microsporidia, parasites and predators

the European strain, then and now, are conducted outside the generally infested area. Because of the flight capabilities of the Asian strain and the expanded potential host range, USDA policy is to eradicate moths exhibiting characteristic traits or genetic markers consistent with the Asian strain wherever feasible–even inside the generally infested area.

Knowledge of the time, location, and extent of an introduction is required to trigger eradication of the Asian strain in the generally infested area. In cases where deductive, circumstantial, or investigative information can be developed about an introduction of uncertain origin, eradication may also be conducted. The goal is to eradicate gypsy moths that exhibit traits characteristic of the Asian strain in a specific area wherever it may occur (within or outside the generally infested area).

Treatments available are the same for both strains, but the timing of application differs. Eradication of the European strain begins with a detection survey that locates isolated infestations, followed by a delimiting survey confirming the presence of established populations and determining the approximate size and geographic extent of the infestation (see Appendix B for survey descriptions). Treatment ensues at the conclusion of the delimiting survey; time from detection to initial treatment is 1 to 2 years.

Treatment for the Asian strain begins the year after detection. Time is not taken to conduct a delimiting survey, as an isolated infestation of the Asian strain could spread significantly because of the female's flight capability, resulting in the need for an even larger eradication project. During the year following detection, the treatment area is determined using the best information available; the area extends beyond where male moths are collected, compensating for the distance females might fly. Delimiting surveys follow treatment, conducted throughout and significantly beyond the treated area.

Population Phases.

Populations of the gypsy moth periodically build to high levels for one or more years, then collapse and remain at low levels for varying periods of time before increasing again. These changes in population levels pass through four phases (Doane and McManus 1981, USDA Forest Service 1989):

Innocuous Phase.

Populations are low and stable. Predation by small mammals and birds and parasitism by other insects appear to keep populations low (Campbell 1976, Elkinton and Leibhold 1990). This phase was undoubtedly the major contributing factor in the 1900 decision to cancel the eradication program.

Release Phase.

Populations build rapidly. While not fully understood, mild winters followed by warm, dry springs and summers may increase survival and lead to population expansion and increase (Campbell and Sloan 1977c).

Outbreak Phase.

Populations reach high levels, and feeding causes widespread moderate-to-heavy defoliation of susceptible hosts. Although predation and parasitism of caterpillars continue, the impact on gypsy moth populations is minor. As the outbreak progresses, the gypsy moth virus--a naturally occurring nucleopolyhedrosis virus--or a fungus (*Entomophaga maimaiga*) may begin to build in the population and contribute to its collapse (Campbell and Sloan 1977c).

Decline Phase.

Populations collapse from overpopulation, starvation, infection by the virus or fungus and decreased reproduction. Males frequently outnumber females in these populations; the other phases exhibit approximately equal numbers of males and females.

Host Plants.

Caterpillars of the European strain eat foliage from a wide variety of trees and shrubs. They prefer oaks, apple, sweetgum, speckled alder, basswood, gray and white birch, poplar, willow and hawthorn (McManus and others 1989). All instars feed on these species; later instars feed on some additional tree species shunned by early instars, such as cottonwood, hemlock, southern white cedar, and the pines and spruces in the eastern United States. The gypsy moth usually does not feed on some plants, including rhododendron, laurel, dogwood, and yellow poplar, although during an outbreak gypsy moth caterpillars will feed on almost all vegetation (McManus and others 1989). Appendix D provides the gypsy moth's feeding preferences for over 700 plant species.

The Asian strain exhibits a broader range of preferred hosts than does the European strain (USDA APHIS 1992). Studies show the Asian strain thrives with greater vigor than the European strain on many of the hosts species present in the United States, with the largest variability in growth rate observed on conifers (Wallner 1994).

E.2 1869 to 1910: Biological Controls Fail.

The European strain of the gypsy moth was considered a curiosity when it first escaped around 1869 from an insectary in Medford, Massachusetts. Public perception of the moth as a problem developed two decades later as the gypsy moth population exploded; citizens soon realized the consequences of allowing the moths to remain uncontrolled:

> In the summer of 1889 it [the gypsy moth] threatened to overrun Medford, Massachusetts. The startled townspeople discovered caterpillars in astounding numbers, swarming through trees, eating leaves, and coating the ground below with

droppings. People swept insects from their sidewalks, porches, and clothes; carried umbrellas to ward off droppings and falling caterpillars; and even wore face nets. The town, unable to deal with the situation, appealed to the state for aid. The striking nature of the infestation and its occurrence in an urban area brought quick response from the commonwealth, and an ambitious effort to deal with the pest was begun. (Dunlap 1980, p. 118)

Gypsy moth, initially assumed native, was first identified as an exotic pest in 1889 (Weseloh 2003c). Control methods included destroying egg masses, burning infested trees and shrubs, banding trees to trap caterpillars, and spraying insecticides. Paris green (copper aceto-arsenate), the first gypsy moth insecticide, was replaced with lead arsenate in 1893 (McManus and McIntyre 1981).

Massachusetts discontinued efforts to eradicate the gypsy moth in 1900, mistakenly considering the project fully successful. The actual reason for the diminished presence of the pest was its entry into the innocuous phase, one of four periodic population phases. A second outbreak in 1906 prompted the Federal government to take action to eliminate the non-native insect. Eradication proved impossible; gypsy moth was already widespread.

Entomologists with the (then) USDA Bureau of Entomology initiated studies to determine the life cycle of the insect and identify natural enemies from Europe for use against the pest. Introduction of identified natural enemies failed to stop the moths, and these biological control efforts were deemed failures. Funding reductions affected even basic research activities:

> Biological control proved to be much more difficult than either the scientists or the public had anticipated. Importing and

establishing the moth's natural enemies was neither simple nor inexpensive. Some of the parasites immediately died in the new environment, others refused to breed, and still others vanished without a trace when released. Some survivors were found to be preying on the moth, but with no noticeable effect on its population. (Dunlap 1980, p. 121).

E.3 1911 to 1939: Chemical Insecticides Gain Favor.

The Bureau of Entomology issued a report in 1911, stating the parameters of effective use of biological controls against the gypsy moth in the United States:

> . . . all fifty of the moth's known European predators [would have to be imported and established], which would require long-term studies of the ecology of the moth and its enemies. (Dunlap 1980, p. 121)

The public, as well as scientists and politicians, quickly realized the successful use of biological controls would require extensive research and funding:

> With the end of hope that natural enemies would control the moth, both state and federal workers fell back on a piecemeal approach. They sought to reduce damage in highly visible and economically important areas— roadsides and towns. (Dunlap 1980, p. 123)

The gypsy moth spread throughout New England. By 1914, the generally infested area included the southern half of New Hampshire, Rhode Island, eastern Connecticut, southern Vermont, and the eastern half of Massachusetts (McManus and McIntyre 1981). The use of chemical insecticides evolved as the favored form of control:

> Their popularity was due in part to … the public's desire for an immediate [visible] solution to [gypsy moth] problems and its reluctance to invest in long-term research that did not promise a certain or immediate return. Chemicals…gave immediate and gratifying visible results. Best of all, they could be used by individual landowners or towns without regard to coordination with other people or jurisdictions…

> In forest spraying, however, chemicals proved ineffective. Better equipment and sprays now made roadside and urban spraying practical…[while] skyrockets and aerial bombs proved interesting but impractical. Spraying from planes or autogiros [early helicopters] seemed promising…[but] the hazards of tall trees, crosswinds, and irregular terrain made spraying difficult, but the most important factor was economic: American forests had too low a return per acre to justify the expense and repeated sprayings that were necessary to control the moth.

> The same economic calculations also doomed another, ecological, control method [that of silvicultural…] replacing stands of susceptible or favored food species with those that were most resistant to the moth's attacks

or less palatable. Unfortunately, this approach, like extensive forest spraying, presupposed a relatively high return per acre, and nothing came of it. (Dunlap 1980, p. 123-124)

The United States Department of Agriculture (USDA), in cooperation with the infested States and Canada, established a barrier zone in 1923, extending from the Canadian border along the Hudson River and Champlain Valleys to Long Island. Gypsy moth infestations east of this barrier were designated for treatment by the States; infestations to the west for eradication. The first major infestation west of the barrier zone occurred in Pennsylvania in 1932. Six years later, the New England hurricane of September 21, 1938, spread the gypsy moth hundreds of miles into new territory. In the following year the barrier zone had become generally infested.

E.4 1940 to 1957: DDT Gets Widespread Use.

Consideration and experimentation of new insecticide controls occurred both before and during World War II. Experimental use of cryolite as a gypsy moth insecticide in Pennsylvania in the 1940s proved ineffective; the most promising new insecticide was a synthetic organic chemical, dichloro-diphenyl-trichlorethane (DDT):

> Even before the end of World War II, American and Canadian scientists were using experimental lots of the new chemical for aerial spraying on northern forests to test DDT against the gypsy moth and the spruce budworm. The results were astounding. Less than a pound of DDT per acre killed almost all the caterpillars, but it did not, apparently, cause any significant damage to wildlife. (Dunlap 1980, p. 124)

Experimental use of DDT in Pennsylvania proved more effective than cryolite, leading to the erroneous conclusion that successful eradication of gypsy moth in the State occurred by 1948. Undetected infestations, however, led to further outbreaks and continued spread (Nichols 1961).

Gypsy moth infestations proliferated in the 1950s, and another barrier zone was set up through the Adirondack plateau in an attempt to prevent spread to the south and west. However, detection of the insect in previously uninfested areas occurred by the mid-1950s. New Jersey, New York, Pennsylvania, and Michigan reported populations, initiating a major Federal effort to eradicate the gypsy moth:

> The first phase, to begin in the spring of 1957, involved aerial spraying to eliminate outlying populations of the moth in New York, Pennsylvania, and Michigan. If these were successful, a second phase would follow, wiping out the main body in New England… The moth's periodic outbreaks caused serious but local damage, and there was no urgent demand to quell the latest one. The only clear rationale was the availability of DDT…

> Spraying began in April 1957 and lasted until June, covering more than 3 million acres in the Northeast with DDT. It brought a storm of criticism from the populace, from scientists, and from local and state officials. Some objected to the nuisance; cars dotted with scum or pools covered with layers of oil [from the carrier used to spray the DDT]. Other effects were more serious: dairy farmers complained that DDT fell on their pastures and passed into the milk, contaminating it. Organic farmers on Long Island also protested, for the

> sprays rendered their crops unsuitable for the special markets… The program also met legal challenge, the first serious environmental litigation against a pest control program…it [proved to be] too controversial for officials and bureaus whose budgets depended on public goodwill. (Dunlap 1980, p. 124-125)

E.5 1958 to the Mid-1980s: Safer Treatments Needed.

During its use, DDT application for gypsy moth control totaled over 12 million acres (4.9 million ha) of forest in nine northeastern States and Michigan (U.S. EPA 1975). Questions concerning the non-target effects of DDT led to its replacement by the carbamate, carbaryl, in the late 1950s. DDT use came to an end soon after publication of Rachel Carson's book *Silent Spring*, in 1962. The Forest Service stopped using DDT in its Eastern Region (Paananen and others 1987). Although considered safer than DDT, in certain formulations carbaryl demonstrated toxicity to honeybees (USDA 1985).

Between 1970 and 1981, suppression of gypsy moth outbreaks was accomplished with aerial applications of broad-spectrum insecticides, including carbaryl and the organophosphate trichlorfon and, to a lesser degree, acephate. These broad-spectrum, nerve-poison insecticides killed not only gypsy moth caterpillars, but many other immature and adult insects in treated areas.

The initiation of research efforts to find effective means of gypsy moth control began in the 1970s, including the use of the gypsy moth nucleopolyhedrosis virus (NPV) and *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*) as biological control agents. Gypchek, registered in 1978, is an insecticide made from NPV. The insect growth-regulator, diflubenzuron, also registered in 1978, offered an attractive alternative with fewer effects on non-target organisms than other chemical insecticides.

The USDA increased exploration for foreign parasites and predators of the gypsy moth in 1971, also funding research on a synthetic pheromone (disparlure) and gypsy moth population dynamics and environmental effects (McManus and McIntyre 1981). Results of this and other research led to the development of non-insecticidal methods, such as mass trapping, mating disruption and the sterile insect technique for use in gypsy moth projects.

Attempts to eliminate the gypsy moth from the United States were abandoned in the 1970s and a two-phase management approach adopted: suppression of outbreaks in the generally infested area and eradication of isolated infestations resulting from inadvertent transport of the insect by people into the uninfested area.

Diflubenzuron and *B.t.k.* largely replaced carbaryl and trichlorfon as the insecticides of choice in cooperative gypsy moth suppression projects by the mid-1980s. Cooperative suppression projects last used trichlorfon in 1984 and carbaryl in 1987 (USDA Forest Service 1994d). Use of broad-spectrum chemical insecticides in cooperative eradication projects ceased in 1989 (USDA APHIS 1992).

E.6 Mid-1980s to the Present: Adoption of Integrated Pest Management.

Integrated pest management (IPM) became the standard approach to gypsy moth suppression and eradication in the 1980s, and continues to this writing. This approach employs the use of various management practices, including the application of chemical and biological insecticides and utilization of non-insecticidal methods.

Up to this point, controls against high-density populations of the gypsy moth were employed in relatively small treatment blocks. Three successive studies began attempts to keep low-density populations from expanding over geographic areas of increasing size.

The Forest Service led Federal, State, and county agencies in an IPM study of a five-county area in Maryland from 1983 to 1987 (Reardon and others 1993). Using geographic information system (GIS) computer technology to collect and store data, this first study accomplished advances in the operational use of controls specific to gypsy moth. An improved formulation of the nucleopolyhedrosis virus (Gypchek) resulted, as well as the first release of sterile eggs.

The second study, conducted in 38 counties along the Appalachian Mountains in Virginia and West Virginia, began in 1987 and concluded in 1992 (USDA Forest Service 1989). Researchers successfully minimized damage in the project area, reducing adverse environmental effects using gypsy-moth-specific treatments in an IPM approach, demonstrating the technical feasibility of slowing the spread of the gypsy moth (USDA Forest Service 1994e).

The third study, a 5-year pilot project started in 1992, utilized the same concepts and methodologies in four States (Virginia, West Virginia, North Carolina, and Michigan), to determine the operational and economic feasibility of a nationwide program to slow the spread of the gypsy moth.

Building upon these three field studies, development and improvement of methods for IPM continued on a national scale for the gypsy moth and for all major forest pests. Participants included the Forest Service National Center for Forest Health Management and other units of the Forest Service, APHIS, Agricultural Research Service and Cooperative State Research, Education, and Extension Service.

Following the issuance of the 1995 Final Environmental Impact Statement "Gypsy Moth Management in the United States: a cooperative approach" and the subsequent signing of the 1996 Record of Decision, USDA implemented a program that included eradication, suppression, and slow-the-spread (STS) projects to control the gypsy moth. An STS pilot project concluded in 1999, leading to the first full-scale projects using STS methodologies operationally in 2000. Eradication, suppression, and STS projects continue to this writing.

E.7 1991 to the Present: Asian Strain Creates Additional Concern.

The Asian strain of the gypsy moth, found for the first time in the United States in 1991, is of concern because females have the ability to fly. This mobility poses the possibility of the Asian strain spreading at an even faster rate than does the European strain. Eradication projects for the Asian strain were conducted in Oregon and Washington in 1992 and 1993; in North Carolina in 1994 and 1995; and in Portland, Oregon, in 2000. A single Asian male found in a survey trap in the Port of Long Beach, California, in 2003 led to the implementation of preventive control projects there in 2004.

Isolated infestations of the European strain continue to be a problem outside of the generally infested area, usually resulting from inadvertent movement of gypsy moth life stages on articles such as cars, campers, outdoor furniture, and nursery stock. Port-of-entry activities to prevent all gypsy moth strains from entering the United States are ongoing (see Appendix B). Surveys using pheromone traps continue nationally to detect introduction and determine if eradication is necessary.

Gypsy Moth Management in the United States: a cooperative approach
Draft Supplemental Environmental Impact Statement

The complete Draft Supplemental Environmental Impact Statement, Gypsy Moth Management in the United States: a cooperative approach, consists of four volumes:

Volume I   Summary
Volume II   Chapters 1-8 and Appendixes A, B, C, D, E
Volume III   Appendixes F, G, H, I
Volume IV   Appendixes J, K, L, M

Lead Agency: Forest Service, U.S. Department of Agriculture (USDA)

Responsible Official: James R. Hubbard, Deputy Chief for State and Private Forestry
                          Sidney R. Yates Federal Building
                          201 14th Street, SW
                          Washington, DC 20250

For More Information**:** Bill Oldland, SEIS Team Leader
                          180 Canfield Street
                          Morgantown, WV 26505

Joint Lead Agency: Animal and Plant Health Inspection Service (APHIS), USDA

Responsible Official: Richard L. Dunkle, Deputy Administrator for Plant Protection and Quarantine
                          4700 River Road
                          Riverdale, MD 20737-1236

For More Information: Weyman P. Fussell, Ph.D., Gypsy Moth Program Coordinator
                          Emergency and Domestic Programs, APHIS
                          4700 River Road, Unit 134
                          Riverdale, MD 20737-1236

Abstract: The USDA Forest Service and Animal and Plant Health Inspection Service are proposing an addition to the gypsy moth management program that was described in the 1995 Environmental Impact Statement--Gypsy Moth Management in the United States: a cooperative approach--and chosen in the 1996 Record of Decision. The agencies are proposing these new treatment options: adding the insecticide tebufenozide, or adding the insecticide tebufenozide and other new treatment(s) that may become available in the future to manage gypsy moths, provided that the other treatment(s) poses no greater risk to human health and nontarget organisms than are disclosed in this Draft SEIS for the currently approved treatments and tebufenozide.

Commenting on this Draft Supplemental Environmental Impact Statement: Reviewers should provide the Forest Service with their comments during the review period of this draft supplemental environmental impact statement. Timely comments will enable the Forest Service to analyze and respond to all of the comments at one time and to use information acquired in the preparation of the final supplemental environmental impact statement, thus avoiding undue delay in the decision making process. Furthermore, the more specific and substantive the comments, the better for reviewers and the agencies alike. Reviewers have an obligation to structure their participation in the National Environmental Policy Act process so that it is meaningful and alerts the agency to the reviewer's position and contentions (Vermont Yankee Nuclear Power Corp. v. NRDC, 435 U.S. 519, 553, 1978). Environmental objections that could have been raised at the draft stage may therefore be forfeited, if not raised until after completion of the final environmental impact statement (Department of Transportation v. Public Citizen, 541 U.S. 752, 764 (2004). Comments on this draft supplemental environmental impact statement should be specific and should address the adequacy of the statement and the merits of the alternatives discussed (40 CFR 1503.3).

Web Site for Draft SEIS: The Draft SEIS is available for viewing at www.na.fs.fed.us/wv/eis

Send Comments to: Bill Oldland, SEIS Team Leader
USDA Forest Service
180 Canfield Street
Morgantown, WV 26505
304-285-1585

Date Comments Must Be Received: See cover letter for the date that comments are due in Morgantown, WV.

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Figure H-1.   (UGA1275009) USDA Forest Service Archives, www.forestryimages.org

Figure I-1.   (UGA1275014) USDA Forest Service Archives, www.forestryimages.org

Appendix F
Bacillus thuringiensis kurstaki (*B.t.k.*) Risk Assessment

Figure F-1. This insecticide mist blower, designed and constructed in 1946 by Quincy Forestry Department, Massachusetts, was used to spray trees in residential areas.

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

Prepared for:

USDA, Forest Service
Forest Health Protection

GSA Contract No. **GS-10F-0082F**
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Task No. **5**

Submitted to:
Dave Thomas, COTR
Forest Health Protection Staff
USDA Forest Service
Rosslyn Plaza Building C, Room 7129C
1601 North Kent Street
Arlington, VA 22209

Submitted by:
Patrick R. Durkin
Syracuse Environmental Research Associates, Inc.
5100 Highbridge St., 42C
Fayetteville, New York 13066-0950
Telephone: (315) 637-9560
Fax: (315) 637-0445
E-Mail: SERA_INC@msn.com
Home Page: www.sera-inc.com

June 8, 2004 (Risk Assessment)
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GENERAL ACRONYMS, ABBREVIATIONS, AND SYMBOLS

a.i.	active ingredient
AEL	adverse-effect level
APHIS	Animal and Plant Health Inspection Service
ARS	Agricultural Research Station
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
B.t.k.	*Bacillus thuringiensis* var. *kurstaki*
BIU	Billions of international units
bw	body weight
cfu	colony forming units
cm	centimeter
DFB	diflubenzuron
EC_{50}	concentration causing 50% inhibition of a process
EC_{100}	concentration causing complete inhibition of a process
EEC	expected environmental concentration
EIS	environmental impact statement
F	female
F_1	first filial generation
FH	Forest Health
FS	Forest Service
FTU	forestry toxic units
g	gram
GC	gas chromatography
GRAS	generally recognized as safe
HQ	hazard quotient
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
i.p.	intraperitoneal
IU	international units
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
K_p	skin permeability coefficient
L	liter
LdNPV	gypsy moth (*Lymantria dispar*) nucleopolyhedrosis virus
lb	pound
LC_{50}	lethal concentration, 50% mortality
LD_{50}	lethal dose, 50% mortality
LD_{95}	lethal dose, 95% mortality
LOAEL	lowest-observed-adverse-effect level
m	meter
M	male
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
MSDS	material safety data sheet
MW	molecular weight
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration

ACRONYMS, ABBREVIATIONS, AND SYMBOLS (*continued*)

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

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

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

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

CONVERSION OF SCIENTIFIC NOTATION

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

EXECUTIVE SUMMARY

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

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

Sensitive terrestrial insects are the only organisms likely to be seriously affected by exposure to *B.t.k.* or its formulations. All sensitive terrestrial insects are lepidoptera and include some species of butterfly, like the endangered Karner blue and some swallowtail butterflies and promethea moths. At the application rates used to control gypsy moth populations, mortality rates among sensitive terrestrial insects are likely to range from approximately 80% to 94% or more. The risk characterization for other wildlife species is unambiguous: under foreseeable conditions of exposure, adverse effects are unlikely to be observed.

In terms of potential human health effects, formulations of *B.t.k.* are likely to cause irritation to the skin, eyes, and respiratory tract; however, serious adverse health effects are implausible. For members of the general public, exposure levels are estimated to be below the functional human NOAEL for serious adverse effects by factors of about 28,000 to 4,000,000 [4 million]. At the extreme upper range of exposure in ground workers, exposure levels are estimated to be below the functional human NOAEL for serious effects by a factor of 25. This assessment is based on reasonably good monitoring data, conservative exposure assumptions, and an aggressive and protective use of the available toxicity data.

PROGRAM DESCRIPTION

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

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

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

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

HUMAN HEALTH RISK ASSESSMENT

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

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

Exposure Assessment – Exposure assessments usually estimate the amount or concentration of an agent to which an individual or population might be exposed via ingestion, dermal contact, or inhalation. The exposure assessments are then compared with toxicity studies based on similar types of exposure—i.e., the dose-response assessment—and then the risk is quantified. The human health risk assessment for *B.t.k.* is unusual in two respects. First, the most directly relevant data used to characterize risk are based on actual applications of *B.t.k.* formulations where exposure is best characterized as an application rate. Second, the apparent lack of a specific mechanism of toxicity for *B.t.k.* makes selecting the most appropriate measure of exposure somewhat arbitrary.

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

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

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

Risk Characterization – The risk characterization regarding exposure to *B.t.k.* and its formulations is generally consistent with that of the previous USDA risk assessment as well as more recent risk assessments conducted by the U.S. EPA and the World Health Organization: *B.t.k.* and its formulations are likely to cause irritation to the skin, eyes, and respiratory tract; however, serious adverse health effects are implausible. Nonetheless, more recent information alters the approach taken to quantifying the risk of exposure-related irritant effects and more serious health effects, thereby affecting the risk characterization. Unlike the previous USDA risk assessment, there is no attempt to quantify the risk of irritant effects. This approach is taken because the threshold for these effects cannot be determined. At application rates similar to those conducted by USDA in programs to control or eradicate the gypsy moth, some members of the general public as well as workers are likely to experience throat irritation, which is the best documented effect in the *B.t.k.* literature on human health effects. Nonetheless, dermal and ocular irritation are also likely effects, although perhaps only at the extreme upper levels of exposure.

B.t.k. applications to control or eradicate the gypsy moth are not expected to cause serious adverse health effects in humans. At the extreme upper range of exposure in ground workers, exposure levels are estimated to be below the functional human NOAEL for serious effects by a factor of 25. For members of the general public, exposure levels are estimated to be below the functional human NOAEL by factors of about 28,000 to 4,000,000 [4 million]. This assessment is based on reasonably good monitoring data, conservative exposure assumptions, and an aggressive and protective use of the available toxicity data. Based on these data, it is not likely that overt signs of toxicity will be observed in any group— ground workers, aerial workers, or members of the general public—exposed to *B.t.k.* as the result of gypsy moth control and eradication programs conducted by the USDA.

There is no documented evidence of a subgroup of individuals who are more sensitive than most members of the general public to *B.t.k.* formulations. According to a recent epidemiology study, asthmatics are not likely to be adversely affected by aerial applications of *B.t.k.* The literature on *B.t.k.* includes one anecdotal claim of a severe allergy to a carbohydrate in a *B.t.k.* formulation; however, neither the claim nor observations of similar effects are substantiated in the available published epidemiology studies. On the other hand, *B.t.k.* formulations are complex mixtures, and the possibility that individuals may be allergic to some of the components in the formulations is acknowledged by a state health service.

Pre-treatment with an influenza virus substantially increased morality in mice exposed to various doses of *B.t.k.* This effect raises concern about the susceptibility of individuals who have influenza or other viral respiratory infections to severe adverse responses to *B.t.k.* exposure. The viral enhancement of bacterial infections is not uncommon and the enhancement of *B.t.k.* toxicity by a viral infection is, in some respects, not surprising. The relevance of this observation to public health cannot be assessed well at this time. No such effects are reported in the epidemiology studies conducted to date. It is, however, not clear that the epidemiology studies would detect such an effect or that such an effect is plausible under the anticipated exposure levels (typical or extreme) used in programs to control the gypsy moth. The viral enhancement of *B.t.k.* toxicity is likely to be an area of further study in the coming years.

ECOLOGICAL RISK ASSESSMENT
Hazard Identification – The hazard identification for mammals is closely related to the hazard identification for the human health risk assessment in that both are based, in part, on numerous standard toxicity studies in experimental mammals. Although *B.t.k.* may persistent in mammals for several weeks after exposure, there is little indication that oral or dermal exposure leads to any serious adverse effects. Most inhalation studies do not suggest a potential for adverse effects even at *B.t.k.* concentrations much greater than those likely to be encountered in the environment. The lack of a positive hazard identification is supported by field studies which demonstrate a lack of adverse effects in populations of mammals after applications of *B.t.k.*

Toxicity studies in birds are limited to standard acute exposures required by U.S. EPA for product registration. The studies all involve either single-dose gavage administration or five daily dose gavage administrations, and none of the studies reports signs of toxicity or pathogenicity at single oral doses up to 3333 mg formulation/kg bw or at multiple oral doses up to 2857 mg formulation/kg bw. Due to the lack of toxicity of *B.t.k.* formulations as well as other *B.t.* strains, the U.S. EPA did not require chronic or reproductive toxicity studies in birds. This apparent lack of the toxicity is supported by numerous field studies in birds. In one field study, a transient decrease in abundance was noted in one species, the spotted towhee (*Pipilo maculatus*). This observation is inconsistent with other field studies on *B.t.k.,* and, according to the investigators, may be an artifact of the study design.

The mechanism of action of *B.t.k.* in lepidoptera is relatively well characterized. *B.t.k.* vegetative cells produce spores and crystals. After the insect consumes the crystals, toxins are formed that attach to the lining of the mid-gut of the insect and rupture the cell walls. The *B.t.k.* spores germinating in the intestinal tract enter the body cavity through the perforations made by the crystal toxins and replicate causing septicemia and eventually death. While various strains of *B.t.* are often characterized as selective pesticides, *B.t.k.* is toxic to several species of target and non-target lepidoptera. Sensitive non-target lepidoptera include larvae of

the Karner blue butterfly, two species of swallowtail butterflies, a promethea moth, the cinnabar moth, and various species of Nymphalidae, Lasiocampidae, and Saturniidae.

While some non-target lepidopteran species appear to be as sensitive as target species to *B.t.k.*, most studies indicate that effects in other terrestrial insects are likely to be of minor significance. There is relatively little information regarding the toxicity of *B.t.k.* or *B.t.k.* formulations to terrestrial invertebrates other than insects. Some oil-based *B.t.k.* formulations may be toxic to some soil invertebrates; however, the toxicity is attributable to the oil in the formulation and not to *B.t.k.* There is no indication that *B.t.k.* adversely affects terrestrial plants or soil microorganisms.

The U.S. EPA classifies *B.t.k.* as virtually non-toxic to fish, and this assessment is consistent with the bulk of experimental studies reporting few adverse effects in fish exposed *B.t.k.* concentrations that exceed environmental concentrations associated with the use of *B.t.k.* in USDA programs. Although there are no data regarding the toxicity of *B.t.k.* or its formulations to amphibians, other strains of *B.t.* appear to have low toxicity to amphibians. The effects of *B.t.k.* on aquatic invertebrates is examined in standard laboratory studies and in numerous field studies. At concentrations high enough to cause decreases in dissolved oxygen or increased biological oxygen demand, *B.t.k.* may be lethal to certain aquatic invertebrates, like *Daphnia magna*. Most aquatic invertebrates, however, seem relatively tolerant to *B.t.k.* This assessment is supported by several field studies that have failed to note remarkable effects in most species after exposures that substantially exceed expected environmental concentrations. As with effects on terrestrial plants, the toxicity of *B.t.k.* to aquatic plants has not be tested.

The U.S. EPA (1998) has raised concerns that some batches of *B.t.* may contain heat labile exotoxins that are toxic to *Daphnia*. The production of these toxins is an atypical event thought to be associated with abnormal or poorly controlled production process. The U.S. EPA requires manufacturers to submit a daphnid study on each new manufacturing process to demonstrate that heat labile exotoxin levels are controlled.

Exposure Assessment – Based on the hazard identification, exposure assessments are presented for three groups: small mammals, terrestrial insects, and aquatic species. While a number of different exposure scenarios could be developed for terrestrial mammals, the only positive hazard identification for *B.t.k.* involves inhalation exposures. As in the human health risk assessment, inhalation exposures of 100 to 5000 cfu/m^3 are used to assess potential risks of serious adverse effects in terrestrial vertebrates. These concentrations are applied to a 20 g mouse and correspond to inhaled doses of 0.00336 to 0.168 cfu/mouse. While there is no basis for asserting that any oral and/or dermal exposures are likely to cause adverse effects in terrestrial vertebrates, an extremely conservative exposure assessment is developed for combined oral (water and vegetation) and dermal (direct spray) exposures that yields an estimated maximum dose of about 184 mg/kg body weight. For terrestrial insects, the toxicity values used to assess the consequences of observing effects is given in units of BIU/ha. Consequently, the exposure assessment for this group is simply the range of application rates used in USDA programs —i.e., about 49 to 99 BIU/ha. For aquatic organisms, toxicity data are expressed in several different units such as mg formulation/L, IU/L, and cfu/L. Based on application rates used in USDA programs and conservative assumptions concerning the depth of water over which *B.t.k.* might be sprayed, concentrations in water would be expected to be at or below 0.24 mg formulation/L. As discussed in the hazard identification, there is no basis for asserting that adverse effects in birds, plants, soil microorganisms, or soil invertebrates other than insects are of plausible concern. Consequently, explicit exposure assessments are not conducted for those groups.

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

Risk Characterization – Terrestrial insects are the only organisms likely to be adversely affected by exposure to *B.t.k.* or its formulations. Separate dose-response curves can be generated for both sensitive and tolerant terrestrial insects. At the application rates used to control gypsy moth populations, mortality rates among sensitive terrestrial insects are likely to range from approximately 80% to 94% or more. All sensitive terrestrial insects are lepidoptera and include some species of butterfly, like the endangered Karner blue and some swallowtail butterflies and promethea moths. For some lepidoptera, sensitivity to *B.t.k.* is highly dependent on developmental stage. This is particularly evident for the cinnabar moth, where late instar larvae are very sensitive to *B.t.k.* and early instar larvae are very tolerant to *B.t.k.* Given the mode of action of *B.t.k.*—i.e., it must be ingested to be highly toxic to the organism— effects on even the most sensitive species will occur only if exposure coincides with a sensitive larval stage of development. In tolerant species, including non-lepidopteran insects and certain larval stages of some lepidoptera, the anticipated mortality rates are much lower (on the order of less than 1% to about 4%). The risk characterization for terrestrial mammals is unambiguous: under foreseeable conditions of exposure, adverse effects are unlikely to be observed. Similarly, based on a very conservative exposure assessment for aquatic species, effects in fish and aquatic invertebrates appear to be unlikely. As discussed in the hazard identification, effects in birds, plants, soil microorganisms, or soil invertebrates other than insects are not of plausible concern. Thus, quantitative risk characterizations for these groups are not conducted. For oil-based formulations of *B.t.k.* (or any other pesticide), effects in some soil invertebrates—i.e., Collembola or earthworms—are plausible.

1. INTRODUCTION

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

This is a technical support document and it addresses some specialized technical areas. Thus, parts of this document may contain information that is difficult for some readers to understand. These technical discussions are necessary to support the review of the document by individuals with specialized training. Nevertheless, an effort is made to ensure that the conclusions reached in the document and the bases for these conclusions can be understood by individuals who do not have specialized training in the chemical and biological sciences. Each major section of the document starts with an overview section that is intended to summarize the technical discussion in a manner that most individuals will understand. In addition, certain technical concepts, methods, and terms common to all parts of the risk assessment are described in plain language in a separate document (SERA 2001). Some of the more complicated terms and concepts are defined, as necessary, in the text.

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

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

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

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

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

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

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

The risk assessment methods used in this document are similar to those used in risk assessments previously conducted for the Forest Service as well as risk assessments conducted by other government agencies. Details regarding the specific methods used to prepare the human health risk assessment are provided in SERA (2001). This document has four chapters, including the introduction, program description, risk assessment for human health effects, and risk assessment for ecological effects or effects on wildlife species. Each of the two risk assessment chapters has four major sections, including an identification of the hazards associated with *B.t.k.* and its commercial formulations, an assessment of potential exposure to the product, an assessment of the dose-response relationships, and a characterization of the risks associated with plausible levels of exposure. These are the basic steps recommended by the National Research Council of the National Academy of Sciences (NRC 1983) for conducting and organizing risk assessments.

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

2. PROGRAM DESCRIPTION

2.1. Overview

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

2.2. Chemical Description and Commercial Formulations

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

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

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

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

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

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

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

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

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

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

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

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

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

2.3. Use Statistics

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

In order to minimize the ecological effects and human health effects of gypsy moth infestations, the USDA adopted various intervention strategies that are roughly categorized as suppression, eradication, and slow the spread (Liebhold and McManus 1999). Suppression efforts are conducted by the USDA Forest Service in areas of well established gypsy moth infestations to combat or interdict periodic gypsy moth population outbreaks. Eradication efforts are conducted by USDA/APHIS to completely eliminate gypsy moth populations in areas where new populations of the gypsy moth are found. Slow the spread, as the name implies, is a program to reduce the expansion of gypsy moth populations from areas of established populations to adjacent non-infested areas.

3. Human Health Risk Assessment

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

3.1.3. Mechanism of Action (Persistence and Pathogenicity)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

3.1.7. Effects on Immune System
Immunotoxicants are chemical agents that disrupt the function of the immune system. Two general types of effects, suppression and enhancement, may be seen and both of these effects are generally regarded as adverse. Agents that impair immune responses (*immune suppression*) enhance susceptibility to infectious diseases or cancer. Enhancement or *hyperreactivity* can give rise to *allergy* or hypersensitivity, in which the immune system of genetically predisposed

individuals inappropriately responds to chemical or biological agents (e.g., plant pollen, cat dander, flour gluten) that pose no threat to other individuals or *autoimmunity*, in which the immune system produces antibodies to self components leading to destruction of the organ or tissue involved.

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

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

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

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

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

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

3.1.8. Effects on Endocrine System
In terms of functional effects that have important public health implications, effects on endocrine function would be expressed as diminished or abnormal reproductive performance. This issue is addressed specifically in the following section (Section 3.1.9). Mechanistic assays are generally used to assess the potential for direct action on the endocrine system (Durkin and Diamond 2002). Neither *B.t.k.* nor any other strain of *B.t.* was tested for activity as an agonist or antagonist of the major hormone systems (e.g., estrogen, androgen, thyroid hormone). Accordingly, all inferences concerning the potential effect of *B.t.* on endocrine function must be based on inferences from standard toxicity studies. As noted in the previous section, U.S. EPA (1998) concludes that there is no basis for asserting that strains of *B.t.* are likely to have an impact on the endocrine system.

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

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

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

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

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

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

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

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

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

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

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

3.1.12. Systemic Toxic Effects from Parenteral Exposure

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

3.1.13. Inhalation Exposure

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

Only two studies (David 1990c; Hernandez et al. 2000) have reported mortality after exposure to *B.t.k.* and both of these studies, while related to inhalation toxicity, involve atypical routes of exposure. Intratracheal instillations of bacteria are analogous to inhalation exposures in that the bacteria is essentially inserted into the lungs. One such study (David 1990c) was conducted on a *B.t.k.* Dipel formulation. As detailed in Appendix 1, toxic responses including death were observed in treated animals and the time-to-clearance (estimated from linear regression) was prolonged. Also, Hernandez et al. (2000) assayed the toxicity of *B.t.k.* after intranasal instillations in mice. This method of dosing is also analogous to inhalation exposures in that the material is deposited in nasal passages and the *B.t.k.* is gradually transported to the lungs by inhalation. Doses of 10^2, 10^4, and 10^6 cfu/mouse caused only local inflamation. A dose of 10^8 cfu/mouse resulted in 80% lethality. The relevance of these two studies to the human health risk assessment is discussed further in Section 3.3 (Dose-Response Assessment).

3.1.14. Impurities

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

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

3.1.15. Inerts

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

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

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

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

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

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

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

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

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

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

3.2.2. General Issues
As discussed in Section 2 and considered further in Section 4.1, the potency of *B.t.k.* is often expressed as BIU or FTU and exposures or application rates are expressed in units of BIU or FTU per acre. Although these units may be meaningful expressions of exposure for the gypsy moth, they are not necessarily or even likely to be a meaningful measures of human exposure. Toxicity to sensitive insects like the gypsy moth is generally attributed to a combination of the delta-endotoxin and the spore coat. These two factors probably account for the potency of the commercial formulations in the bioassays used to determine the BIU/mg of commercial product. Unlike the gut of the gypsy moth, which has a high pH (that is, the gut is alkaline or basic) the stomach of most mammals, including humans, has a low pH (that is, the stomach contents are acidic). Thus, the delta-endotoxin is not toxicologically significant for humans.

Another commonly used measure of exposure to *B.t.k.* formulations is *colony forming units* or cfu. When *B.t.k.* formulations are applied, either by aerial spray or ground spray, one or more viable spores contained in droplets or particulates is suspended in the air and deposited on sprayed surfaces. These droplets may be collected, either by air sampling or direct deposition, onto various types of filters. The filters are then cultured in a nutrient medium under conditions conducive to bacterial growth. As the bacteria grow, visible masses of bacteria, referred to as colonies, appear on the media. In the case of monitoring *B.t.k.* formulations, some of the colonies will be *B.t.k.* and some colonies will be other endogenous bacteria. Microscopic examination, differential culturing, or other methods may be used to determine the number of colonies that are *B.t.k.* By this general method, the number of cfu per unit of surface area or volume of air, depending on the sampling method, may be determined. Each cfu can be formed from a droplet or particulate that contains one or more viable spores. Thus, the number of cfu per unit of surface area or volume of air does not correspond directly to the number of viable spores per unit of surface area or volume of air. Dilution methods can be used to determine the number of viable spores (Palmgren et al. 1986).

The significance of cfu as a measure of human exposure is limited. As discussed in Section 3.1.3, there is little indication that *B.t.k.* is a human pathogen. Consequently, the number of viable spores, albeit an important measure of exposure for the gypsy moth, does not appear to be toxicologically significant to humans. In this respect, cfu like BIU are of limited significance. Nonetheless, at least for short-term exposures, cfu can be used as a practical measure of relative exposure to a *B.t.k.* formulation.

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

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

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

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

3.2.3. Workers

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

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

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

3.2.4. Members of the General Public

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

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

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

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

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

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

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

3.3.2. Existing Guidelines
Dose-response assessments for the systemic toxic effects of most pesticides are based on an RfD, an estimate of a dose or exposure that is not likely to induce substantial adverse effects in humans. The RfD, in turn, is typically based on a NOAEL (no observed adverse effect level) divided by an uncertainty factor. Risk is then characterized as a hazard quotient (HQ) which is the estimated level of exposure divided by the RfD. If the HQ is below unity—i.e., the exposure is less than the RfD —there is no credible risk. If the HQ is above unity, risk is characterized based on dose-response or dose-severity relationships.

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

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

infectivity/pathogenicity studies normally required ... be waived in the future as long as product identity and manufacturing process testing data indicate there is no mammalian toxicity associated with the strain (U.S. EPA, 1998, p. 11).

The application methods suggest that the potential for eye, dermal and inhalation exposure to mixers, loaders and applicators does exist. ... However, because of a lack of mammalian toxicity, the risk from occupational exposure is minimal ... the health risk [to the general public] *is expected to be negligible due to: (1) The lack of toxicological concerns associated with Bacillus thuringiensis, and (2) Bacillus thuringiensis has been used as a pesticide for approximately 50 years with no known adverse effects* (U.S. EPA, 1998, p. 14).

The World Health Organization reaches a similar conclusion:

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

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

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

3.3.3. Human Data

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

include generalized dermal irritation (dry or itchy skin and chapped lips), irritation to the throat, and respiratory irritation (cough or tightness). Moreover, the overall incidence of all symptoms combined was increased significantly among the workers, compared with the controls .

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

While it is beyond the scope of this risk assessment to discuss Bonferroni's correction in detail, it should be noted that Bonferroni's correction is conservative—i.e., it will reduce the number of false positive associations. In terms of a risk assessment, Bonferroni's correction may be viewed as anti-conservative in that the presence of a large number of trivial comparisons could obscure statistically and biologically significant results for a subset of important comparisons. Thus, as discussed by Perneger (1998), judgement and an assessment of biological plausibility must be exercised in the application of Bonferroni's correction. Specifically for this risk assessment of *B.t.k.*, these judgements are discussed further in Section 3.2.5). When Bonferroni's correction is applied to the data from Cook (1994) in Table 3-4, none of the effects are statistically significant at p<0.007; however, skin irritation (p≈0.0077) and throat irritation (p≈0.0079) are marginally significant.

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

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

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

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

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

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

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

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

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

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

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

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

3.3.4. Animal Data
As noted in Section 3.1.13 and summarized in Appendix 1, there is essentially no information indicating that inhalation exposure to *B.t.k.* will cause serious adverse health effects. Extremely severe inhalation exposures that coat the test species with commercial formulations of *B.t.k.* are associated with decreased activity, discolored lungs, and other effects but not mortality. Although the animal data are consistent with data regarding human exposure *B.t.k.*, the animal studies are all based on single concentrations and cannot be used in a meaningful dose-response assessment.

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

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

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

In terms of the human health risk assessment, these data are not directly useful. Furthermore, the route of exposure (intranasal instillation) makes any use of these data somewhat tenuous. Concern with the use of this atypical route of exposure in a dose-response assessment is exacerbated because the Hernandez et al. (2000) study does not specify whether or not the instillations were adjusted to a constant volume. If the installations were not adjusted to a constant volume, it is possible that could be observed in animals with a compromised respiratory tract (i.e., because of viral infection) because of volumetric bronchial obstruction or a combination of bronchial obstruction and *B.t.k.*

Notwithstanding these reservations, the Hernandez et al. (1999, 2000) studies provide the best dose-response data available in experimental mammals. Table 3-7 provides dose conversions that may be valuable in further exploring the useful of these data. In Table 3-7, the first column indicates the cfu/mouse from the studies by Hernandez et al. (1999, 2000)and the second column provides the estimated concentration of *B.t.k.* required to achieve the cfu/mouse dose in a 1-hour exposure. This value is calculated as cfu/mouse divided by the estimated breathing rate (m^3/hour) of a 20 g mouse.

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

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

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

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

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

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

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

3.3.5. Values Used for Risk Characterization

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

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

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

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

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

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

3.4. RISK CHARACTERIZATION
3.4.1. Overview
The risk characterization for *B.t.k.* and its formulations is consistent with the risk characterization in the previous USDA risk assessment as well as more recent risk assessments conducted by the U.S. EPA and the World Health Organization: *B.t.k.* and its formulations are likely to cause irritant effects to the skin, eyes, and respiratory tract; however, serious adverse health effects are not of plausible concern. Nevertheless, the approach used to quantify risk for irritant effects and more serious health effects is different, based on recent information regarding *B.t.k.* exposure.

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

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

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

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

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

3.4.2. Irritant Effects

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

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

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

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

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

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

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

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

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

3.4.3. Serious Adverse Effects

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION
4.1.1. Overview.
The hazard identification for mammals is closely related to the hazard identification for the human health risk assessment in that both are based, in part, on numerous standard toxicity studies in experimental mammals. Although *B.t.k.* may persistent in mammals for several weeks after exposure, there is little indication that oral or dermal exposure leads to any serious adverse effects. Most inhalation studies do not suggest a potential for adverse effects even at *B.t.k.* concentrations much greater than those likely to be encountered in the environment. The lack of a positive hazard identification is supported by field studies which demonstrate a lack of adverse effects in populations of mammals exposed to applications of *B.t.k.* Nonetheless, there are data to suggest that extremely high concentrations of *B.t.k.* in air might pose a hazard.

Toxicity studies in birds are limited to standard acute exposures required by U.S. EPA for product registration. The studies all involve either single-dose gavage administration or five daily-dose gavage administrations, and none of the studies reports signs of toxicity or pathogenicity at single oral doses up to 3333 mg formulation per kg bw or at multiple oral doses up to 2857 mg formulation per kg bw. Due to the lack of toxicity of *B.t.k.* formulations as well as other *B.t.* strains, the U.S. EPA did not require chronic or reproductive toxicity studies in birds. The apparent lack of *B.t.k.* toxicity is supported by numerous field studies in birds. In one field study, a transient decrease in abundance was noted in the spotted towhee (*Pipilo maculatus*). This observation is inconsistent with other field studies on *B.t.k.,* and, according to the investigators, may be an artifact of the study design.

The mechanism of action of *B.t.k.* in lepidoptera is relatively well characterized. *B.t.k.* vegetative cells produce spores and crystals. After the insect consumes the crystals, toxins are formed that attach to the lining of the mid-gut of the insect and rupture the cell walls. The *B.t.k.* spores germinate in the intestinal tract and enter the body cavity through the perforations made by the crystal toxins. The bacteria replicate in the body cavity, causing septicemia and eventual death. While various strains of *B.t.* are often characterized as selective pesticides, *B.t.k.* is toxic to several species of target and non-target lepidoptera. Sensitive non-target lepidoptera include larvae of the Karner blue butterfly, two species of swallowtail butterflies, a promethea moth, the cinnabar moth, and various species of Nymphalidae, Lasiocampidae, and Saturniidae.

While some non-target lepidopteran species appear to be as sensitive as target species to *B.t.k.*, most studies indicate that effects in other terrestrial insects are likely to be of minor significance. There is relatively little information regarding the toxicity of *B.t.k.* or *B.t.k.* formulations to terrestrial invertebrates other than insects. Some oil-based *B.t.k.* formulations may be toxic to some soil invertebrates; however, the toxicity is attributable to the oil in the formulation and not to *B.t.k.* There is no indication that *B.t.k.* adversely affects terrestrial plants or soil microorganisms.

The U.S. EPA classifies *B.t.k.* as virtually non-toxic to fish, and this assessment is consistent with the bulk of experimental studies reporting few adverse effects in fish exposed *B.t.k.* concentrations that exceed environmental concentrations associated with the use of *B.t.k.* in USDA programs. Although there are no data regarding the toxicity of *B.t.k.* or its formulations to amphibians, other strains of *B.t.* appear to have low toxicity to amphibians. The effects of *B.t.k.* on aquatic invertebrates is examined in standard laboratory studies and in numerous field studies. At concentrations high enough to cause decreases in dissolved oxygen or increased

biological oxygen demand, *B.t.k.* may be lethal to certain aquatic invertebrates, like *Daphnia magna*. Most aquatic invertebrates, however, seem relatively tolerant to *B.t.k.* This assessment is supported by several field studies that have failed to note remarkable effects in most species after exposures that substantially exceed expected environmental concentrations. As with effects on terrestrial plants, the toxicity of *B.t.k.* to aquatic plants has not been tested.

U.S. EPA (1998) raises concerns that some batches of *B.t.* may contain heat labile exotoxins that are toxic to *Daphnia*. The production of these toxins is an atypical event thought to be associated with abnormal or poorly controlled production process. The U.S. EPA requires manufacturers to submit a daphnid study on each new manufacturing process to demonstrate that heat labile exotoxin levels are controlled.

4.1.2. Toxicity to Terrestrial Organisms.
4.1.2.1. Mammals –The hazard identification for mammals is closely related to the hazard identification for the human health risk assessment (see Section 3.1) in that both are based, in part, on numerous standard toxicity studies in experimental mammals (Appendix 1). As discussed in Section 3.1 and summarized inAppendix 1, *B.t.k.* may persistent—i.e., may survive and be recovered—in mammals for several weeks after exposure; however, there is little indication that oral or dermal exposure leads to serious adverse health effects. Most inhalation studies do not suggest a potential for adverse effects even at *B.t.k.* concentrations much greater than those likely to be encountered in the environment. The lack of a positive hazard identification is supported by field studies in which no adverse effects were observed in populations of mammals exposed to *B.t.k.* applications of (Belloq et al. 1992; Innes and Bendell 1989). Nonetheless, as discussed in the human health risk assessment (see Section 3.3.4), there are data to suggest that extremely high air concentrations of *B.t.k.* in air might pose a hazard.

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

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

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

4.1.2.2. Birds – Toxicity studies in birds are limited to standard acute exposures required by U.S. EPA for product registration. The studies all involve either single-dose gavage administration (Beavers et al. 1988a) or five daily-dose gavage administrations (Beavers 1991b; Lattin et al. 1990a,b,c,d,e,f,g), and none of the studies reports signs of toxicity or pathogenicity at single oral doses up to 3333 mg formulation/kg bw or at multiple oral doses up to 2857 mg formulation/kg bw (Appendix 2). Due to the lack of evidence regarding acute toxicity in birds exposed to *B.t.k.* formulations or other *B.t.* strains, the U.S. EPA did not require chronic or reproductive toxicity studies in birds.

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

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

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

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

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

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

Herms et al. (1997) demonstrate the only dose-response relationships after applications of *B.t.k.* to both target and non-target lepidoptera. In this study, the toxicity of Foray 48B was assayed in larvae of both the gypsy moth and the Karner blue butterfly, an endangered species of butterfly indigenous to the northern United States (Minnesota to New Hampshire). Bioassays in both species involved applications of Foray 48B to vegetation (wild lupine leaves for the Karner blue and white oak leaves for the gypsy moth) at treatment levels equivalent to either 30 to 37 BIU/ha per ha (low dose) or 90 BIU/ha (high dose). A negative control consisted of untreated vegetation. The insect larvae (either 1st or 2nd instar for the Karner blue and 2nd instar for the gypsy moth) were placed on the vegetation 7 to 8 hours after treatment and allowed to feed for 7 days. Survival rates for Karner blue larvae were: 100% for controls, 27% at the 30 to 37 BIU/ha treatment rate, and 14% at the 90 BIU treatment rate. Survival rates for gypsy moth larvae were: 80% for controls; 33% for low-dose treatment, and 5% for high-dose treatment. As detailed further in the dose-response assessment (Section 4.3), the differences between the gypsy moth and Karner blue do not appear to be substantial and the Karner appears to be as sensitive as the target species to *B.t.k.*

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4.2. EXPOSURE ASSESSMENT
4.2.1. Overview.
The exposure assessment for the ecological risk assessment on *B.t.k.* are summarized in Table 4-3. Exposure assessments, based on the hazard identification, are presented for three groups: small mammals, terrestrial insects, and aquatic species. Although numerous exposure scenarios could be developed for terrestrial mammals, the only positive hazard identification for *B.t.k.* involves inhalation exposures. The ecological risk assessment uses inhalation exposure levels of 100 to 5000 cfu/m^3, which is the same range used in the human health risk assessment, to assess potential risks of serious adverse effects in terrestrial vertebrates. These concentrations are applied to a 20 g mouse and correspond to inhaled doses of 0.00336 to 0.168 cfu/mouse. While there is no credible basis for asserting that terrestrial invertebrates are likely to have adverse effects after oral or dermal exposure to *B.t.k.*, an extremely conservative exposure assessment is developed for combined oral (water and vegetation) and dermal (direct spray) exposures that yields an estimated maximum dose of approximately 184 mg/kg body weight. For terrestrial insects, the toxicity values used to assess the consequences of observing effects is given in units of BIU/ha. Consequently, the exposure assessment for this group is simply the range of application rates used in USDA programs—i.e., about 49 to 99 BIU/ha. For aquatic organisms, toxicity data are expressed in several different units, including mg formulation/L, IU/L, and cfu/L. Based on application rates used in USDA programs and conservative assumptions concerning the depth of water over which *B.t.k.* might be sprayed, concentrations in water are expected to be less than or equal to 0.24 mg formulation/L. As discussed in the hazard identification, there is no basis for concern about adverse effects in birds, plants, soil microorganisms or invertebrates ,other than insects, exposed to *B.t.k.* Hence, explicit exposure assessments for these groups are not conducted.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

$$Y = a + bx$$

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

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

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

$$Y = a + bx + cS$$

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4.4. RISK CHARACTERIZATION
4.4.1. Overview.
An overview of the risk characterization for *B.t.k.* is presented in Table 4-6. The only organisms that are likely to be affected by *B.t.k.* or *B.t.k.* formulations are terrestrial insects. Separate dose-response curves can be generated for both sensitive and tolerant terrestrial insects. At the application rates used to the control of the gypsy moth, the expected mortality rates for sensitive terrestrial insects range from about 80% to 94%. All sensitive terrestrial insects are comprised of lepidoptera, including some species of butterflies, like the endangered Karner blue, and some swallowtail butterflies and promethea moths. In some cases, lepidopteran sensitivity to *B.t.k.* is highly dependent on developmental stage. This is particularly true for the cinnabar moth, with late instar larvae being as sensitive as target species to *B.t.k.* and early instar larvae being among the most tolerant lepidoptera. Given the mode of action of *B.t.k.*—i.e., it must be ingested in order to be highly toxic—effects on even the most sensitive species are anticipated only when species are in a sensitive larval stage at the time of or shortly after *B.t.k.* application. Much lower mortality rates (on the order of less than 1% to about 4%) are anticipated in tolerant species, including non-lepidopteran insects and certain lepidoptera at a particular stage of development. The risk characterization for terrestrial mammals is unambiguous: under foreseeable conditions of exposure, adverse effects are unlikely. Similarly, based on a very conservative exposure assessment for aquatic species, effects in fish and aquatic invertebrates appear to be unlikely. As discussed in the hazard identification, effects in birds, plants, soil microorganisms or invertebrates other than insects appear to be of no plausible concern. Thus, quantitative risk characterizations for these groups are not conducted. For oil-based formulations of *B.t.k.* (or any other pesticide), effects are plausible for some soil invertebrates —i.e., Collembola or earthworms.

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

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

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

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

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

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

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

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

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

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

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

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

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

5. LIST OF STUDIES CONSULTED

Addison JA. 1995. Persistence and nontarget effects of Bacillus thuringiensis in soil: a review. Can J For Res. 23:2329-2342

Addison JA; Holmes SB. 1995. Effect of two commercial formulations of *Bacillus thuringiensis* subsp. *kurstaki* Dipel 8L and Dipel 8AF on the collembolan species *Folsomia candida* in a soil microcosm study. Bull Environ ContamToxicol. 55(5):771-778

Addison JA; Holmes SB. 1996. Effect of two commercial formulations of *Bacillus thuringiensis* subsp. *kurstaki* on the forest earthworm *Dendrobaena octaedra*. Can J For Res. 26:1594-1601

Aer'aqua Medicine Ltd. 2001. Health Surveillance following Operation Ever Green: A Programme to eradicate the white-spotted tussock moth from the eastern suburbs of Auckland. Report to the Ministry of Agriculture and Forestry, New Zealand, May 2001

Aer/aqua Medicine Ltd. 2001. Health Surveillance following Operation Ever Green: A Programme to eradicate the white-spotted tussock moth from the eastern suburbs of Auckland. Report to the Ministry of Agriculture and Forestry, New Zealand, May 2001

Akiba Y. 1986. Microbial ecology of Bacillus thuringiensis: VI. Germination of *Bacillus thuringiensis* spores in the soil. Appl Entomol Zool. 21:76-80

Akiba Y. 1991. Assessment of rainwater-mediated dispersion of field-sprayed *Bacillus thuringiensis* in the soil. Appl Entomol Zool. 26:477-483

Appel HM; McCarthy WJ. 1999. Hostplant incompatibility with biological control: tannins, bt, and the gypsy moth. U. S. Department of Agriculture Competitive Research Grant

Aronson AI; Han ES; Mcgaughey W; Johnson D. 1991. The solubility of inclusion proteins from *Bacillus thuringiensis* is dependent upon protoxin composition and is a factor in toxicity to insects. Appl Environ Microbiol. 57:981-986

Atkins E. 1991a. Bee adult toxicity dusting test evaluating the comparative acute contact and stomach poison toxicity of BT III dry flowable.(*Bacillus thuringiensis* var. kurstaki) to honey bee worker adults: Lab Project No. 91/838. Unpublished study prepared by Univ. California, Riverside. 13 p. MRID 41983301

Atkins E. 1991b. Bee adult toxicity dusting test evaluating the comparative acute contact and stomach poison toxicity of BT I dry flowable. (*Bacillus thuringiensis* var. *kurstaki*) to honey worker adults: Lab Project No. 91/836. Unpublished study prepared by Univ. California. Riverside. 13 p. MRID 41983501

Auckland District Health Board. 2002. Health Risk Assessment of the 2002 Aerial Spray Eradication Programme for the Painted Apple Moth in Some Western Suburbs of Auckland. A Report to the Ministry of Agriculture and Forestry. Prepared by: Public Health Service, Auckland District Health Board, Auckland, New Zealand. Available at: http://www.adhb.co.nz/akphp/Services/AppleMoth/PAM%20HRA%20-%20with%20maps.pdf

Barridge B. 1990a. Delta BT-product identity: Lab Project No. DBP 1989-100. Unpublished study prepared by Delta Biological Products. 16 p. MRID 41751101

Barridge B. 1990b. Delta BT-formation of unintentional ingredients: Lab Project No. DBP 1989-102. Unpublished study prepared by Delta Biological Products. 8 p. MRID 41751102

Barridge B. 1990c. Delta BT-analysis of samples: Lab Project No. DBP 1989-103. Unpublished study prepared by Delta Biological Products. 20 p. MRID 41751103

Barridge B. 1990d. Delta BT-certification of limits: Lab Project No. DBP 1989-104. Unpublished study prepared by Delta Biological Products. 5 p. MRID 41751104

Barridge B. 1990e. Delta BT–manufacturing process: Lab Project No. DBP 1989-101. Unpublished study prepared by Delta Biological Products, Inc. 8 p. MRID 42080101

Barridge B. 1990f. Delta BT-physical and chemical properties: Lab Project No. DPB 1989-105. Unpublished study prepared by Delta Biological Products, Inc. 6 p. MRID 42080102

Barry JW; Skyler PJ; Teske ME; Rafferty JA; Grim BS. 1993. Predicting and measuring drift of *Bacillus thuringiensis* sprays. Env Toxicol Chem. 12(11):1977-1989.

Bassett J; Watson M. 1999a. Acute ORAL TOXICITY (LD 50) study in rats with DiPel technical powder: Lab Project No. 7363-98-0123-TX-001: 7636-98-0123-TX-000. Unpublished study prepared by Ricerca Inc. 45 p. MRID No. 44791605

Bassett J; Watson M. 1999b. Primary dermal irritation study in albino rabbits with DiPel technical powder: Lab Project Nos. 7635-98-0124-TX-001: 7635-98-0124: 7635-98-0124-TX-000. Unpublished study prepared by Ricerca, Inc. 52 p. MRID 44791607

Beavers J. 1991a. ABG-6305: An avian oral pathogenicity and toxicity in the bobwhite: Lab Project No. 161-117. Unpublished study prepared by Wildlife International Ltd. 21 p. MRID 41974804

Beavers J. 1991b. ABG-6305: An avian oral pathogenicity and toxicity in the bobwhite: Lab Project No. 161-118. Unpublished study prepared by Wildlife International Ltd. 20 p. MRID 41974805

Beavers J; Jaber M. 1987. SAN 418 SC 62 *Bacillus thuringiensis* Tenebrionis: An avian acute oral ld50 pathogenicity study in the mallard : Study No. 131-132. Unpublished study performed by Wildlife International Ltd. 19 p. MRID 40497409

Beavers J; Jaber M. 1987. SAN 418 SC 62 *Bacillus thuringiensis* Tenebrionis: An avian intraperitoneal injection pathogenicity study in the mallard: Study No. 131-133. Unpublished study performed by Wildlife International Ltd. 19 p. MRID 40497410

Beavers J; Smith G. 1990a. An avian oral pathogenicity and toxicity study in the mallard: Lab Project No. 297-106. Unpublished study prepared by Wildlife International Ltd. 19 p. MRID 41751108

Beavers J; Smith G. 1990b. An avian oral pathogenicity and toxicity study in the bobwhite: Lab Project No. 297-105. Unpublished study prepared by Wildlife International Ltd. 21 p. MRID 41751109

Beavers J; Smith G. 1991a. *Bacillus thuringiensis* var. Israelensis, strain NB31: An avian oral pathogenicity and toxicity study in the mallard: Lab Project No. 254-125. Unpublished study prepared by Wildlife International Ltd. 25 p. MRID 41842702

Beavers J; Smith G. 1991b. *Bacillus thuringiensis* var. Israelensis, strain NB31: An avian oral pathogenicity and toxicity study in the bobwhite: Lab Project No. 254-124. Unpublished study prepared by Wildlife International Ltd. 25 p. MRID 41842703

Beavers J; Clauss B; Jaber M. 1988a. *Bacillus thuringiensis* Strain EG2348: An Avian Acute Oral LD50 Pathogenicity Study in the Bobwhite. Wildlife International Ltd. Project No.: 235-120. Unpublished study prepared by Wildlife International Ltd. 19 p. MRID 40898807

Beavers J; Clauss B; Jaber M. 1988b. *Bacillus thuringiensis* strain EG2424: An avian acute oral LD50 pathogenicity study in the mallard. Wildlife International Lt. Project No. 235-122. Unpublished study prepared by Wildlife International Ltd. 19 p. MRID 40951107

Beavers J; Clauss B; Jaber M. 1988b. *Bacillus thuringiensis* strain EG2424: An avian acute oral LD50 pathogenicity study in the mallard. Wildlife International Ltd. Project No. 235-122. Unpublished study prepared by Wildlife International Ltd. 19 p. MRID No. 40951107

Beevers M. 1990. Effects of *Bacillus thuringiensis* subp. kurstaki on the insect egg parasitoid, *Trichogramma pretiosum*: Final Report: Lab Project No. CAR/103-90. Unpublished study prepared by California Agricultural Research, Inc. 41 p. MRID 41443409

Behle RW; Mcguire MR; Gillespie RL; Shasha BS. 1997. Effects of alkaline gluten on the insecticidal activity of *Bacillus thuringiensis*. J Econ Entomol. 90:354-360

Behle RW; Mcguire MR; Shasha BS. 1997. Effects of sunlight and simulated rain on residual activity of *Bacillus thuringiensis* formulations. J Econ Entomol. 90:1560-1566

Bell J. 1994. Personal communication. Agriculture Canada. Telephone conversation with Patrick R. Durkin, February 25, 1994

Bell JL; Whitmore RC. 1997. Bird populations and habitat in *Bacillus thuringiensis* and Dimilin-treated and untreated areas of hardwood forest. Am Midl Nat. 137:239-250

Bellantoni D; Grimstead S; Roberts C; et al. 1991a. Delta BT: A toxicity and pathogenicity test with the rainbow trout (*Oncorhynchus mykiss*): Final Report: Lab Project No. 297A-101. Unpublished study prepared by Wildlife International Ltd. 25 p. MRID 41899101

Bellantoni D; Grimstead S; Holmes C; et al. 1991b. Delta BT: A toxicity and pathogenicity test with the cladocern (*Daphnia magna*): Final Report: Lab Project No. 297A-104. Unpublished Study prepared by Wildlife International Ltd. 28 p. MRID 41899102

Bellantoni D; Grimstead S; Roberts C; et al. 1991c. Delta BT: A toxicity and pathogenicity test with the grass shrimp (*Palaemonetes pugio*): Final Report: Lab Project Number: 297A-102. Unpublished study prepared by Wildlife International Ltd. 24 p. MRID 41899103

Bellantoni D; Grimstead S; Roberts C; et al. 1991d. Delta BT: A toxicity and pathogenicity test with the sheepshead minnow (*Cyprinodon variegatus*): Final Report: Lab Project No. 297A-103. Unpublished study prepared by Wildlife International Ltd. 25 p. MRID 41899104

Belloq MI; Bendell JF; Cadogan BL. 1992. Effects of the insecticide *Bacillus thuringiensis* on *Sorex cinereus* (masked shrew) populations, diet, and prey selection in jack pine plantation in northern Ontario. Can J Zool. 70:505-510

Ben-Dyke R; Hogan GK; Hoffman CA; et al. 1981. An acute inhalation toxicity and infectivity study of Thuricide 32-B in therRat: Project No. 80-7472. Unpublished study received Mar 8, 1982 under 11273-2; prepared by Bio/dyanamics, Inc., submitted by Sandoz, Inc.--Crop Protection, San Diego, Calif.; CDL:246967-C. MRID 00096529

Benz G; Altweg A. 1975. Safety of *Bacillus thuringiensis* for earthworms. J Invert Pathol. 26:125-126

Berg N. 1989. Acute dermal toxicity study in rabbits with SP 408, PPQ 2585 in support of registration of Novodor technical: Lab Project I.D.: 13188. Unpublished study prepared by Novo-Nordisk A/S, Enzyme Toxicology Laboratory. 16 p. MRID 41412705

Berg N. 1990. *Bacillus thuringiensis* var. kurstaki, batch BBB 0073: Acute intravenous toxicity/pathogenicity study in rats. Enzyme Toxicology Laboratory; June 19, 1990; 108 p. Sponsored by Novo Nordkisk A/S, Bagsvaerd, Denmark

Berg N. 1991a. Eye irritation study in rabbits with the end product Foray 48B, batch BBN 6056. Enzyme Toxicology Laboratory; April 15, 1991; 19 p. Sponsored by Novo Nordisk A/S, Bagsvaerd, Denmark

Berg N. 1991b. Eye irritation study in rabbits with the end product Foray 48B, batch BBN 6057: in support of Formula Amendment of Foray 48B. Enzyme Toxicology Laboratory; April 18, 1991; 19 p. Sponsored by Novo Nordisk A/S, Bagsvaerd, Denmark

Berg N; Kiehr B. 1991. Eye irritation study in rabbits with the end product Foray 48B, batch BBN 6057. Enzyme Toxicology Laboratory; February 26, 1991; 19 p. Sponsored by Novo Nordisk A/S, Bagsvaerd, Denmark

Berg N; Sorensen E; Overholt J. 1991. Summary of acute toxicology in support of formula amendment of Foray 48B:. Lab Project No. NOVO/FFCFA/VOL1. Unpublished study prepared by Novo Nordisk A/S & Novo Nordisk Bioindustrials, Inc. 21 p. MRID 41884301

Bernier RL; Gannon DJ; Moser GP; Mazzarello M; Griffiths MM; Guest PJ. 1990. Development of a novel B.t. strain for the control of forestry pests. Brighton Crop Protection Conference, Pests and Diseases. 1:245-251. (Cited in Glare and O'Callaghan 2000)

Berstein IL; Bernstein JA; Miller M; Tierzieva S; Sernstein DI; Jummus Z; Selgrade MJK; Doerfler DL; Seligy VL. 1999. Immune responses in farm workers after exposure to *Bacillus thuringiensis* pesticides. Environ. Hlth Perspect. 107:575-582

Betz FS; Hammond BG; Fuchs RL. 2000. Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. Regul Toxicol Pharmacol. 32(2):156-173

Birkhold D. 1999. Fermentation manufacturing process for *Bacillus thuringiensis* spp. kurstaki at Abbott Laboratories. Lab Project No. BTKMAN-28R. Unpublished study prepared by Abbott Laboratories. 13 p. MRID 45136503

Bishop AH; Johnnson C; Perant M. 1999. The safety of *Bacillus thuringiensis* to mammals investigated by oral and subcutaneous dosage. World J. Microbiol Biotechnol. 15:375-380

Blackmore H. 2003. Painted Apple Moth Eradication Campaign, West Auckland. Interim Report of the Community-based health and incident monitoring of the aerial spray programme: January - December 2002. Report dated February, 2003. Available at: http://www.geocities.com/no_spray/index2.html

Boeri R. 1991. Chronic toxicity of ABG-6305 to daphnid: *Daphnia magna*: Lab Project No. 90162-A. Unpublished study prepared by Resource Analysts, Inc. 47 p. MRID 41974802

Boxenbaum J; D'Souza R. 1990. Interspecies pharmacokinetic scaling, biological design and neoteny. Adv Drug Res. 19:139-195

Broderick NA; Goodman RM; Raffa KF; Handelsman J. 2000. Synergy between zwittermicin a and *Bacillus thuringiensis* subsp. *kurstaki* against gypsy moth (Lepidoptera: lymantriidae). Environ Entomol. 29:101-107

Buckner CH; Ray DGH; McLeod BB. 1973. The effects of pesticides on small forest vertebrates of the Spruce Woods Provincial Forest, Manitoba. Manitoba Entomol. 7:37-45

Buckner CH; Kingsbury PD; McLeod BB; Mortensen KL; Ray DGH. 1974. Impact of aerial treatment on non-target organisms, Algonquin Park, Ontario, and Spruce Woods, Manitoba, Section F. *In*: Evaluation of Commercial Preparations of *Bacillus Thuringiensis* with and Without Chitinase Against Spruce Budworm. .Ottawa, Ontario, Canadian Forestry Service, Chemical Control Research Institute, pp 1-72.

Cameron EA; Reeves RM. 1990. Carabidae (Coleoptera) associated with gypsy moth, (Lymantria dispar (L.) Lepidopter:Lymantriidae), populations subjected to *Bacillus thuringiensis* Berliner treatments in Pennsylvania. Can Entomol. 122:123-129

Cannon GE; Krize JW. 1975. TH 6040 egg to egg reproduction study in fathead minnows treated at .1, .05, .025, .0125, to .00675 ppm:. Laboratory No. 5E 6094. Unpublished study received Feb 10, 1976 under 6G1744; prepared by Cannon Laboratories, Inc., submitted by Thompson-Hay. MRID 00038616

Cerf D. 1990a. Susceptibility of four orders of insects (lepidoptera, diptera, coleoptera, and orthoptera) to technical grade active ingredients (TGAI) and tenebrionis (strain SA10): Final Report No. 90/03/12. Unpublished study prepared by Sandoz Crop Protection Corp. 40 p. MRID 41441516

Cerf D. 1990b. Susceptibility of four orders of insects (lepidoptera, diptera, coleoptera, and orthoptera) to technical grade active ingredients (TGAI) and tenebrionis (strain SA10): Lab Project No. 90/03/12. Unpublished study prepared by Sandoz Crop Protection Corp. 40 p. MRID 41441625

Cerstiaens A; Verleyen P; Van Rie J; et al. 2001. Effect of *Bacillus thuringiensis* cry1 toxins in insect hemolymph and their neurotoxicity in brain cells of lymantria dispar. Appl Environ Microbiol. 67(9):3923-3927

Chandler G. 1990a. Chronic toxicity of *Bacillus thuringiensis* var. Israelensis technical material to the benthic harpacticoid copepod, *Amphiascus minutus* under static conditions: Report No. USC-SPH-2-90: Abbott Lab-VTP-12. Unpublished study prepared by Univ. South Carolina. MRID 41439010

Chandler G. 1990b. Chronic toxicity of *Bacillus thuringiensis* var. kurstaki technical material to the benthic harpacticoid copepod, *Amphiascus minutus* under static conditions: Toxicity test report: Lab Project No. USC-SPH-1-90. Unpublished study prepared by Univ. South Carolina. MRID 41443408

Chen C; Macuga R. 1990a. Plasmid profile of *Bacillus thuringiensis* subsp. Aizawai, strain SA2: Final Report No. 90/02/03E. Unpublished study prepared by Sandoz Crop Protection Corp. 40 p. MRID 41441507

Chen C; Macuga R. 1990b. Plasmid profile of *Bacillus thuringiensis* subsp. Israelensis, strain SA3: Final Report No. 90/02/03C. Unpublished study prepared by Sandoz Crop Protection Corp. 40 p. MRID 41441508

Chen C; Macuga R. 1990c. Plasmid profile of *Bacillus thuringiensis* subsp. Israelensis, strain SA3A: Final Report No.90/02/03D. Unpublished study prepared by Sandoz Crop Protection Corp. 40 p. MRID 41441509

Chen C; Macuga R. 1990d. Flagella antigen serotyping of *Bacillus thuringiensis* subsp. Aizawai, strain SA2: Final Report No. 90/02/12E. Unpublished study prepared by Sandoz Crop Protection Corp. 23 p. MRID 41441510

Chen C; Macuga R. 1990e. Flagella antigen serotyping of *Bacillus thuringiensis* subsp. Israelensis, strain SA3: Final Report No. 90/02/12C. Unpublished study prepared by Sandoz Crop Protection Corp. 23 p. MRID 41441511

Chen C; Macuga R. 1990f. Flagella antigen serotyping of *Bacillus thuringiensis* subsp. Israelensis, strain SA3A: Final Report No. 90/02/12D. Unpublished study prepared by Sandoz Crop Protection Corp. 23 p. MRID 41441512

Chen C; Macuga R. 1990g. Description of endotoxin proteins produced by *Bacillus thuringiensis* subsp. Aizawai, strain SA2: Final Report No. 90/02/21C. Unpublished study prepared by Sandoz Crop Protection Corp. 30 p. MRID 41441517

Chen C; Macuga R. 1990i. Description of endotoxin proteins produced by *Bacillus thuringiensis* subsp. Israelensis, strain SA3A: Final Report No. 90/02/21F. Unpublished study prepared by Sandoz Crop Protection Corp. 27 p. MRID 41441519

Chen C; Macuga R. 1990j. Plasmid profile of *Bacillus thuringiensis* subsp. tenebrionis strain SA10: Lab Project No: 90/02/03F. Unpublished study prepared by Sandoz Crop Protection Corp. 40 p. MRID 41441613

Chen C; Macuga R. 1990k. Plasmid profile of *Bacillus thuringiensis* subsp. *kurstaki* strain SA11001C98-1-1: Lab Project No. 90/0203A. Unpublished study prepared by Sandoz Crop Protection Corp. 40 p. MRID 41441614

Chen C; Macuga R. 1990l. Plasmid profile of *Bacillus thuringiensis* subsp. *kurstaki* strain INT-15-313: Lab Project No. 90/02/03. Unpublished study prepared by Sandoz Crop Protection Corp. 40 p. MRID 41441615

Chen C; Macuga R. 1990m. Plasmid profile of *Bacillus thuringiensis* subsp. *kurstaki* strain SA12: Lab Project No. 90/02/03B. Unpublished study prepared by Sandoz Crop Protection Corp. 40 p. MRID 41441616

Chen C; Macuga R. 1990n. Flagella antigen serotyping of *Bacillus thuringiensis* subsp. tenebrionis strain SA10: Lab Project No. 90/02/12F. Unpublished study prepared by Sandoz Crop Protection Corp. 23 p. MRID 41441617

Chen C; Macuga R. 1990o. Flagella antigen serotyping of *Bacillus thuringiensis* subsp. *kurstaki* strain SA11001C98-1-1: Lab Project No. 90/02/12A. Unpublished study prepared by Sandoz Crop Protection Corp. 23 p. MRID 41441618

Chen C; Macuga R. 1990p. Flagella antigen serotyping of *Bacillus thuringiensis* subsp. *kurstaki* strain SA11001C98-1-1: Lab Project No. 90/02/12A. Unpublished study prepared by Sandoz Crop Protection Corp. 23 p. MRID 41441619

Chen C; Macuga R. 1990q. Flagella antigen serotyping of *Bacillus thuringiensis* subsp. *kurstaki* strain SA12: Lab Project No. 90/02/12B. Unpublished study prepared by Sandoz Crop Protection Corp. 23 p. MRID 41441620

Chen C; Macuga R. 1990r. Description of endotoxin proteins produced by *Bacillus thuringiensis* subsp. *kurstaki* strain INT-15-313: Lab Project No. 90/02/21A. Unpublished study prepared by Sandoz Crop Protection Corp. 30 p. MRID 41441626

Chen C; Macuga R. 1990s. Description of endotoxin proteins produced by *Bacillus thuringiensis* subsp. *kurstaki* strain -1-1: Lab Project No. 90/02/21. Unpublished study prepared by Sandoz Crop Protection Corp. 30 p. MRID 41441627

Chen C; Macuga R. 1990t. Description of endotoxin proteins produced by *Bacillus thuringiensis* subsp. *kurstaki* strain SA12: Project No. 900221B. Unpublished study prepared by Sandoz Crop Protection Corp. 30 p. MRID 41441628

Chen C; Macuga R. 1990u. Description of endotoxin proteins produced by *Bacillus thuringiensis* subsp. tenebrionis strain SA10: Lab Project No. 90/02/21D. Unpublished study prepared by Sandoz Crop Protection Corp. 25 p. MRID 41441629

Chen C; Macuga R. 1990v. Description of Endotoxin Proteins Produced by *Bacillus thuringiensis* subsp. Israelensis, Strain SA3: Final Report: Final Report No. 90/02/21E. Unpublished study prepared by Sandoz Crop Protection Corp. 27 p. MRID 41441518

Chen C; Macuga R; Cerf D. 1990a. Insecticidal toxins produced by *Bacillus thuringiensis* subsp. Aizawai, strain SA2. I. Effect of autoclaving: Final Report No. 90/01/31E. Unpublished study prepared by Sandoz Crop Protection Corp. 19 p. MRID 41441520

Chen C; Macuga R; Cerf D. 1990b. Insecticidal toxins produced by *Bacillus thuringiensis* subsp. Israelensis, strain SA3. I. Effect of autoclaving: Final Report No. 90/01/31. Unpublished study prepared by Sandoz Crop Protection Corp. 19 p. MRID 41441521

Chen C; Macuga R; Cerf D. 1990c. Insecticidal toxins produced by *Bacillus thuringiensis* subsp. Israelensis, strain SA3A. I. Effect of autoclaving: Final Report No. 90/01/31D. Unpublished study prepared by Sandoz Crop Protection Corp. 19 p. MRID 41441522

Chen C; Cerf D; Sjolander A; et al. 1990d. Insecticidal toxins produced by *Bacillus thuringiensis* subsp. Aizawai, strain. II. Concentration of â-exotoxin: Final Report No. 90/02/07E. Unpublished study prepared by Crop Protection Corp. 36 p. MRID 41441523

Chen C; Cerf D; Sjolander A; et al. 1990e. Insecticidal Toxins produced by *Bacillus thuringiensis* subsp. Israelensis, strain SA3. II. Concentration of â-exotoxin: Final Report No. 90/02/07C. Unpublished study prepared by Sandoz Crop Protection Corp. 36 p. MRID 41441524

Chen C; Cerf D; Sjolander A; et al. 1990f. Insecticidal toxins produced by *Bacillus thuringiensis* subsp. Israelensis, strain SA3A. II. Concentration of â-exotoxin: Final Report No. 90/02/07D. Unpublished study prepared by Sandoz Crop Protection Corp. 36 p. MRID 41441525

Chen C; Macuga R; Cerf D. 1990g. Insecticidal toxins produced by *Bacillus thuringiensis* Subsp. Tenebrionis strain SA10. I. Effect of autoclaving: Final Report: Lab Project No. 90/01/31F. Unpublished study prepared by Sandoz Crop Protection Corp. 19 p. MRID 41441605

Chen C; Macuga R; Cerf D. 1990h. Insecticidal toxins produced by *Bacillus thuringiensis* subsp. *kurstaki* strain SA11001C98-1-1. I. Effect of sutoclaving: Final Report: Lab Project No. 90/01/31A. Unpublished study prepared by Sandoz Crop Protection Corp. 19 p. MRID 41441606

Chen C; Macuga R; Cerf D. 1990i. Insecticidal toxins produced by *Bacillus thuringiensis* subsp. *kurstaki* strain INT-15-313. I. Effect of autoclaving: Final Report: Lab Project No. 90/01/31. Unpublished study prepared by Sandoz Crop Protection Corp. 19 p. MRID 41441607

Chen C; Macuga R; Cerf D. 1990j. Insecticidal toxins produced by *Bacillus thuringiensis* subsp. *kurstaki* strain SA12. I. Effect of autoclaving: Lab Project No. 90/01/31B. Unpublished study prepared by Sandoz Crop Protection Crop. 19 p. MRID 41441608

Chen C; Cerf D; Sjolander A; et al. 1990k. Insecticidal toxins produced by *Bacillus thuringiensis* subsp. *kurstaki* strain SA11001C98-1-1. II. Concentratration of â-exotoxin: Lab Project No. 90/02/07A. Unpublished study prepared by Sandoz Crop Protection Corp. 36 p. MRID 41441630

Chen C; Cerf D; Sjolander A; et al. 1990l. Insecticidal toxins produced by *Bacillus thuringiensis* subsp. *kurstaki* strain INT-15-313. II. Concentration of â-exotoxin: Lab Project No. 90/02/07. Unpublished study prepared by Sandoz Crop Protection Corp. 36 p. MRID 41441631

Chen C; Cerf D; Sjolander A; et al. 1990m. Insecticidal toxins produced by *Bacillus thuringiensis* subsp. tenebrionis strain SA10. II. Concentration of â-exotoxin: Lab Project No. 90/02/07F. Unpublished study prepared by Sandoz Crop Protection. Corp. 36 p. MRID 41441632

Chen C; Cerf D; Sjolander A; et al. 1990n. Insecticidal toxins produced by *Bacillus thuringiensis* subsp. *kurstaki* strain SA12. II. Concentration of â-exotoxin: Lab Project No. 90/02/07B. Unpublished study prepared by Sandoz Crop Protection Corp. 36 p. MRID 41441633

Chen CH; Ding HC; Chang TC. 2001. Rapid identification of bacillus cereus based on the detection of a 28.5-kilodalton cell surface antigen. J Food Prot. 64(3):348-354

Cheon HM; Kim HJ; Kang SK; Seo SJ. 1997. Effects of *Bacillus thuringiensis* delta-endotoxin on insect fat body structure. Korean J Bio Sci. 1(3):507-513

Christensen K. 1990a. Vectobac technical material (*Bacillus thuringiensis* var. Israelensis): Infectivity and pathogenicity to bluegill sunfish (*Lepomis macrochirus*) during a 30-day static renewal test: Final Report: SLI Report 90-2-3228; SLI Study 2439.0889.6104.158. Unpublished study prepared by Springborn Laboratories, Inc. 55 p. MRID 41439007

Christensen K. 1990b. Vectobac technical material (*Bacillus thuringiensis* var. Israelensis): Infectivity and pathogenicity to rainbow trout (*Oncorhynchus mykiss*) during a 32-day static renewal test: Final Report: SLI Report 90-2-3242; SLI Study 2439.0889.103.157. Unpublished study prepared by Springborn Laboratories, Inc. 55 p. MRID 41439008

Christensen K. 1990c. Dipel Technical material (*Bacillus thuringiensis* var. kurstaki): Infectivity and pathogenicity to bluegill sunfish (*Lepomis macrochirus*) during a 32-day static renewal test: Lab Project No. 2439. 0889. 6108. 158. Unpublished study prepared by Springborn Laboratories, Inc. 53 p. MRID 41443405

Christensen K. 1990d. Dipel technical material (*Bacillus thuringiensis* var. kurstaki): Infectivity and pathogenicity to rainbow trout (*Oncorhynchus mykiss*) during a 32-day static renewal test: Lab Project No. 2469.0889.6107.157; 90-2-3219. Unpublished study prepared by Springborn Laboratories, Inc. 57 p. MRID 41443406

Christensen K. 1990e. Vectobac technical material (*Bacillus thurigiensis* var. Iraelensis): Infectivity and pathogenicity to sheep head minnow (*Cyprinodon variegatus*) during a 30-day static renewal test: Lab Project Report No.90-4-3288; Study No. 2439.889.6105.160. Unpublished study prepared by Springborn Laboratories, Inc. 57 p. MRID 41540401

Christensen K. 1990f. Vertobac technical material (*Bacillus thuringiensis* var. Israelensis): Infectivity and Pathogenicity to grass shrimp (*Palaemonetes vulgaris*) during a 31-day static renewal test: Lab Project Report No.90-5-3339; Study No. 2439.0889.6106.161. Unpublished study prepared by Springborn Laboratories, Inc. 50 p. MRID 41540402

Christensen K. 1990g. Dipel technical material (*Bacillus thuringiensis* var. kurstaki): Infectivity and pathogenicity to sheepshead minnow (*Cyprinodon variegatus*) during a 30-day static renewal test: Lab Project No. 90-5-3317: 2439. 0889. 6110. 160. Unpublished study prepared by Springborn Laboratories, Inc. 57 p. MRID 41540801

Christensen K. 1990h. Dipel technical material (*Bacillus thuringiensis* var. kurstaki): Infectivity and pathogenicity to grass shrimp (*Palaemonetes vulgaris*) during a 30-day static renewal test: Lab Project Nos. 90-5-3337: 2439.0889.6109.161. Unpublished study prepared by Springborn Laboratories, Inc. 50 p. MRID 41540802

Christensen K. 1990i. *Bacillus thuringiensis* var. kurstaki: Infectivity and pathogenicity to rainbow trout (*Oncorhynchus mykiss*) during a 31-day static renewal test in support of reregistration of Biobit Flowable Concentrate: Lab Project No. 90/8/3412. Unpublished study prepared by Springhorn Laboratories, Inc. 54 p. MRID 41657009

Christensen K. 1990j. *Bacillus thuringiensis* var. Israelenisis: Infectivity and pathogencity to rainbow trout (*Oncorhynchus mykiss*) during a 32-day static renewal test: Lab Project No. 90-8-3459: 12262.1289.6102.157. Unpublished study prepared by Springborn Laboratoreis, Inc. 50 p. MRID 41980105

Christensen K. 1991a. *Bacillus thuringiensis* var. Israelensis: Infectivity and pathogenicity to bluegill sunfish (*Lepomis macrochirus*) during a 30-day static renewal test: Final Report: Lab Project Nos. 90-8-3460: 12262.1289.6103.158. Unpublished Study prepared by Springborn Laboratories, Inc. 61 p. MRID 41842704

Christensen K. 1991b. *Bacillus thuringiensis* var. Israelensis: Infectivity and pathogenicity to grass shrimp (*Palaemonetes vulgaris*) during a 30-day static renewal test: Final Report: Lab Project Nos. 90-10-3499: 12262.1289.6106.161. Unpublished Study prepared by Springborn Laboratories, Inc. 50 p. MRID 41842706

Christensen K. 1991c. CGA-237218 Technical material: Infectivity and pathogenicity to rainbow trout (*Oncorhyncus mykiss*) during a 32-day static renewal test: Lab Project No. 90-6-3363. Unpublished study prepared by Springborn Labs, Inc. 52 p. MRID 41994315

Christensen K. 1991d. CGA-237218: Chronic toxicity to daphnids (*Daphnia magna*) under static renewal conditions: Lab Project No. 90-7-3385. Unpublished study prepared by Springborn Labs, Inc. 90 p. MRID 41994316

Christensen K. 1991e. CGA-237218: Infectivity and pathogenicity to sheepshead minnow (*Cyprinodon variegatus*) during a 30-day static renewal test: Lab Project No. 90-8-3439. Unpublished study prepared by Springborn Labs, Inc. 50 p. MRID 41994317

Christensen K. 1991f. CGA-237218 technical material: infectivity and pathogenicity to grass shrimp (*Palaemonetes vulgaris*) during a 30-day static renewal test: Lab Project No. 90-6-3445. Unpublished study prepared by Springborn Labs, Inc. 48 p. MRID 41994318

Ciba-Geigy Corp. 1991. Submission of toxicity and product chemistry data in support of registration of Agree insecticide and Ciba-Geigy Technical 237218. Transmittal of 22 studies. MRID 41994300

Clydesdale FM. 1997. Food Additives: Toxicology, Regulation, and Properties. CRC Press, Boca Raton, Florida. CD-ROM Database

Coddens M. 1990a. Dipel FMU: Product chemistry based on *Bacillus thuringiensis*, subspecies *kurstaki* (ATCC-SD-1275) as an active ingredient: Lab Project No. ABBOTT/LAB-FMU-02. Unpublished study prepared by Abbott Laboratories. 92 p. MRID 41435402

Coddens M. 1990b. Vectobac technical powder..product chemistry based on *Bacillus thuringiensis*, subspecies *israelensis*,strain AM65-52 (ATCC-SD-1276) as the active ingredient: Lab Project Nos. Abbott Lab-VTP-03; 910-8902. Unpublished study prepared by Abbott Laboratories. 186 p. MRID 41439002

Coddens M; Cooper R. 1990. Product analysis: product chemistry based on *Bacillus thuringiensis*, subspecies kurstaki. (ATCC-SD-1275 as the active ingredient: Lab Project No. Abbott Lab-FMU-02. Unpublished study prepared by Abbott Laboratories. 23 p. MRID 41435401

Cook GJ. 1994. Bacillus thuringiensis *kurstaki* exposure in ground-spray workers. Major Paper Submitted in Partial Fulfillment of MHSc Degree, (Community Medicine) in the Department of Health Care and Epidemiology, Faculty of Medicine, The University of British Columbia.

Cooke BJ; Regniere J. 1996. An object-oriented, process-based stochastic simulation model of *Bacillus thuringiensis* efficacy against spruce budworm, *Choristoneura fumiferana* (Lepidoptera: tortricidae). Int J Pest Manag. 42:291-306

Cooke BJ; Regniere J. 1999. Predictability and measurability of *Bacillus thuringiensis* efficacy against spruce budworm (Lepidoptera:tortricidae). Environ Entomol. 28:711-721

Cozzi E. 1993a. Intraperitoneal and subcutaneous injection tests with ABG-6345 technical powder: Final Report: Lab Project No. 6345-85K-1. Unpublished study prepared by Abbott Labs. 29 p. MRID 42750401

Cozzi E. 1993b. Intraperitoneal and subcutaneous injection tests with ABG-6346 technical powder. (*Bacillus thuringiensis* subsp. *aizawai*): Final Report: Lab Project No. 6346-85K-1. Unpublished study prepared by Abbott Labs. 28 p. MRID 42791301

Crecchhio C; Stotzky G. 1998. Insecticidal activity and biodegradation of the toxin from *Bacillus thuringiensis* subsp. *kurstaki* bound to humic acids from soils. Soil Biol Biochem. 30:463-470

Crecchio C; Stotzky G. 1996. Binding of the insecticidal proteins from *Bacillus thuringiensis* on humic acids: toxicity and biodegradation. 96th ASM General Meeting, Session 49, 395

Crickmore N; Zeigler DR; Feitelson J; Schnepf E; van Rie J; Lereclus D; Baum J; Dean DH. 1998. Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. Microbiol Mol Biol Rev. 62:807-813

Cunningham JC; Brown KW; Scarr T; Fleming RA; Burns T. 1996. Aerial spray trials with nuclear polyhedrosis virus and *Bacillus thuringiensis* of gypsy moth (Lepidoptera: lymantriidae) in 1994: II. Impact one year after application. Proc Entomol Soc Ontario. 127:37-43

Curtin F; Schulz P. 1998. Multiple correlations and Bonferroni's correction. Biol Psychiatry. 44(8):775-777

Cuthbert JA; Jackson D. 1991. Foray 48B FC: Acute oral toxicity (limit) test in rats in support of formula amendment of Foray 48B. Inveresk Research International, Ltd. Report No. 6902; 17 p. Sponsored by Novo Nordisk A/S, Bagsvaerd, Denmark

Damgaard DH. 1995. Diarrhoeal enterotoxin production by strains of *Bacillus thuringiensis* isolated from commercial *Bacillus thuringiensis*-based insecticides. FEMS Immunol Med Microbiol. 12:245-250

Damgaard PH; Larsen HD; Hansen BM; Bresciani J; Jorgensen K. 1996. Enterotoxin-producing strains of *Bacillus thuringiensis* isolated from food. Lett Appl Microbiol. 23: 146-150

Damgaard PH; Granum PE; Bresciani J; Torregrossa MV; Eilenberg J; Valentino L. 1997a. Characterization of *Bacillus thuringiensis* isolated from infections in burn wounds. FEMS Immunol Medl Microbiol. 18:47-53

Damgaard PH; Hansen BM; Pedersen JC; Eilenberg J. 1997b. Natural occurrence of *Bacillus thuringiensis* on cabbage foliage and in insects associated with cabbage crops. J Appl Microbiol. 82(2):253-258

David R. 1990a. Acute oral toxicity/pathogenicity study of Vectobac technical material. (*Bacillus thuringiensis* var. Israelensis) in rats: Final Report: Lab Study No. G-7264.222. Unpublished study prepared by Microbiological Associates, Inc. 61 p. MRID 41439003

David R. 1990b. Acute oral toxicity/pathogenicity study of Dipel technical material. (*Bacillus thuringiensis* var. *kurstaki*) in rats: Lab Project No. G-7239.222. Unpublished study prepared by Microbiological Associates Inc. 54 p. MRID 41443401

David R. 1990c. Acute pulmonary toxicity/pathogenicity study of Dipel technical material. (*Bacillus thuringiensis* var. *kurstaki* in rats: Lab Project No. G-7239. 001. Unpublished study prepared by Microbiological Associates Inc. 66 p. MRID 41443402

David R. 1990d. Acute pulmonary toxicity/pathogenicity study of Vectobac technical material. (*Bacillus thuringiensis* var. israelensis) in rats: Lab Project No. G-7264.225. Unpublished study prepared by Microbiological Associates Inc. 3 p. MRID 41487401

Drobniewski FA. 1994. A Reveiw: The safety of Bacillus species as insect vector control agents. J Appl Bacteriol. 76:101-109

Drummond J; Kotze AC; Levot GW; Pinnock DE. 1995. Increased susceptibility to *Bacillus thuringiensis* associated with pyrethroid resistance to bovicola (Damalinia) ovis (Phthiraptera mallophaga: possible role of monooxygenases. J Econ Entomol. 88:1607-1610

Dubois NR; Dean DH. 1995. Synergism between Cryia insecticidal crystal proteins and spores of *Bacillus thuringiensis*, other bacterial spores, and vegetative cells against *Lymantria dispar* (Lepidoptera: lymantriidae) larvae. Environ Entomol. 24:1741-1747

Duphar BV. 1990. Submission of product chemistry data in support of *Bacillus thuringiensis* registration standard. Transmittal of 4 studies. MRID 41429600

Durkin PR. 1994. Comparison and Summary of Human Health Risk Assessments for the USDA Control and Eradication Programs. In Proceedings of the 1994 Annual Gypsy Moth Review, D.H. Hilburn, K.J.R. Johnson, and A.D. Mudge (Eds). USDA, Salem, Oregon. pp. 170-182

Durkin PR; Diamond G. 2002. Neurotoxicity, Immunotoxicity, and Endocrine Disruption with Specific Commentary on Glyphosate, Triclopyr, and Hexazinone. Final Report. SERA TR 01-43-08-04a dated January 30, 2002. Available at www.fs.fed.us/foresthealth/pesticide/risk.htm

Ecogen Inc. 1988. Submission of chemistry and toxicity data in support of Foil Oil Flowable insecticide. Transmittal of 10 studies. MRID 41270301

Ecogen Inc. 1988. Product chemistry data submitted to support the registration of EG2349 (*Bacillus thuringiensis*). Transmittal of 1 study. MRID 40729900

Ecogen Inc. 1989. Submission of data in support of registration of Foil Oil Flowable Bioinsecticide: Toxicity studies. Transmittal of 8 studies. MRID 41308600

Eidt DC. 1985. Toxicity of bacillus-thuringiensis-var-*kurstaki* to aquatic insects. Can Entomol. 117:829-838

Eisenbeis G; Lenz R; Heiber T. 1999. Organic residue decomposition: the minicontainer-system a multifunctional tool in decomposition studies. Environ Sci Pollut Res Int. 6(4):220-224

Elliott LJ; Sokolow R; Heumann M; Elefant SL. 1988. An exposure characterization of a large scale application of a biological insecticide, *Bacillus thuringiensis*. Appl Ind Hyg. 3:119-122

Entwistle PF; Cory JS; Bailey MJ; Higgs S. 1993. *Bacillus thuringiensis*, An Environmental Biopesticide: Theory and Practice. John Wiley and Sons, Chichester, England. 311 pp.

Eyal J. 1999. Manufacturing process description for *Bacillus thuringiensis*. Lab Project No. 011990-A. Unpublished study prepared by Thermo Trilogy Corp. 270 p. MRID 44807401

Fares NH; El-Sayed AK. 1998. Fine structural changes in the ileum of mice fed on delta endotoxin-treated potatoes and transgenic potatoes. Nat Toxins. 6(6):219-233

Ferry E. 1990a. Intraperitoneal injection test with Vectobac technical powder: Lab Project No. VTP/TE-05. Unpublished study prepared by Abbott Laboratories. 7 p. MRID 41590302

Ferry E. 1990b. Intraperitoneal and subcutaneous injection tests with ABG-6305 technical powder: Lab Project No. 85K-11/90. Unpublished study prepared by Abbott Laboratories. 6 p. MRID 41722507

Fisher R; Rosner L. 1959. Toxicology of the microbial insecticide, Thuricide. J Agric Food Chem. 7:686-688

Fitch W; Sjolander A; Abrera B. 1990. Determination of Delta Endotoxin in end-use *Bacillus thuringiensis* subsp. *kurstaki* products: Lab Project No. 90/03/01. Unpublished study prepared by Sandoz Crop Protection Corp. 193 p. MRID 41789701

Fletcher JS; Nellessen JE; Pfleeger TG. 1994. Literature review and evaluation of the EPA food-chain (Kenega) nomogram, an instrument for estimating pesticide residues on plants. Environ Toxicol Chem. 13(9):1383-1391

Fortin C; Lapointe D; Charpentier G. 1986. Susceptibility of brook trout (*Salvelinus fontalis*) fry to a liquid formulation of *Bacillus thuringiensis* serova. *israelensis* (Teknas) used for blackfly control. Can J Fish Aquat Sci. 43:1667-1670

Fowler J. 1989a. Physical properties of SA-2 technical grade active ingredient: Final Report No. 89/11/30E. Unpublished study prepared by Sandoz Crop Protection Corp. 32 p. MRID 41441526

Fowler J. 1989b. Physical properties of SA-3 technical grade active ingredient. (TGAI): Final Report No. 89/11/30A. Unpublished study prepared by Sandoz Crop Protection Corp. 32 p. MRID 41441527

Fowler D. 1989c. Physical properties of Certan: Final Report No. 89/11/30D. Unpublished study prepared by Sandoz Crop Protection Corp. 32 p. MRID 41441528

Fowler J. 1989d. Physical properties of Teknar: Final Report No. 89/11/30. Unpublished study prepared by Sandoz Crop Protection Corp. 32 p. MRID 41441529

Fowler J. 1989e. Physical properties of Teknar HPD: Final Report No. 89/11/30B. Unpublished study prepared by Sandoz Crop Protection Corp. 32 p. MRID 41441530

Fowler J. 1989f. Physical properties of SA-3A technical grade active ingredient. (TGAI): Final Report No. 89/11/30C. Unpublished study prepared by Sandoz Crop Protection Corp. 32 p. MRID 41441531

Fowler J. 1989g. Physical properties of 313 1. 5B Dust: Lab Project No. 89/11/30K. Unpublished study prepared by Sandoz Crop Protection Corp. 29 p. MRID 41441634

Fowler J. 1989h. Physical properties of Trident II: Lab Project No. 89/11/30F. Unpublished study prepared by Sandoz Crop Protection Corp. 32 p. MRID 41441635

Fowler J. 1989i. Physical properties of SA-11 spray dried technical concentrate. (SDTC): Lab Project No. 89/11/30M. Unpublished study prepared by Sandoz Crop Protection Corp. 29 p. MRID 41441636

Fowler J. 1989j. Physical properties of 313 spray dried technical concentrate. (SDTC): Lab Project No. 89/11/30J. Unpublished study prepared by Sandoz Crop Protection Corp. 29 p. MRID 41441637

Fowler J. 1989k. Physical properties of SA-10 technical grade active ingredient. (TGAI): Lab Project No. 89/11/30G. 32 p. MRID 41441639

Fowler J. 1989l. Physical properties of SA-11 technical concentrate 360: Final Report: Lab Project No. 89/11/30L. Unpublished prepared by Sandoz Crop Protection Corp. 29 p. MRID 41441640

Fowler J. 1989m. Physical properties of SA-12 technical grade active ingredient: Final Report: Lab Project No. 89/11/30H. Unpublished study prepared by Sandoz Crop Protection Corp. 32 p. MRID 41441641

Fowler J. 1990n. Physical properties of Thuricide 64LV: Lab Project No. 89/11/30I. Unpublished study prepared by Sandoz Crop Protection Corp. 32 p. MRID 41441638

Frankenhuyszen KV; Fast PG. 1989. Susceptibility of three coniferophagous *Choristoneura* species (Lepidoptera: Tortricidae) to *Bacillus thuringiensis* var. *kurstaki*. J Econ Entomol. 82:193-196

Frankenhuyszen KV; Milne R; Brousseau R; Masson L. 1992. Comparative toxicity of the DH-1 and NRD-12 strains of *Bacillus thuringiensis* susp. *kurstaki* to defoliating forest lepidoptera. J Invert Pathol. 59:149-154

Gaddis, PK. 1987. Secondary effects of BT spray on avian predators: the reproductive success of chickadees-1987. Oregon Department of Agriculture, Plant Division Report, Salem. 19 p. (Cited in USDA 1995)

Gaddis, PK; Corkran CC. 1986. Secondary effects of BT spray on avian predators: the reproductive success of chestnut-backed chickadees. Oregon Department of Agriculture, Plant Division Report 86-03, Salem; 20 p. (Cited in USDA/FS 1995)

Gatehouse AM; Ferry N; Raemaekers RJ. 2002. The case of the monarch butterfly: A verdict is returned. Trends Genet. 18(5):249-251

Glare TR; O'Callaghan M. 2000. *Bacillus thuringiensis*: Biology, Ecology and Safety. John Wiley and Sons, Ltd., Chichester, England. 350 pp.

Green M; Heumann M; Sokolow R; et al. 1990. Public health implications of the microbial pesticide *Bacillus thuringiensis*: An epidemiological study, Oregon, (USA) 1985-86. Am J Pub Hlth. 80:848-852

Gujar GT; Kalia V; Kumari A. 2001. Effect of sublethal concentration of *Bacillus thuringiensis* var. *kurstaki* on food and developmental needs of the American bollworm, *Helicoverpa armigera* (Hubner). Ind J Exp Biol. 39(11):1130-1135

Hadley WM; Burchiel SA; McDowell TD; et al. 1987. Five-month oral (diet) toxicity/infectivity study of *Bacillus thuringiensis* insecticides in sheep. Fund Appl Toxicol. 8:236-242

Haile FJ; Kerns DL; Richardson JM; Higley LG. 2000. Impact of insecticides and surfactant on lettuce pH and yield. J Econ Entomol. 93:788-794

Hammond PC; Grimble DG. 1997. Distribution of a northern fauna of noctuidae in the mountains of Oregon. J Lepidopterists' Soc. 51(1):7-101

Harde t. 1990a. *Bacillus thuringiensis kurstaki*: acute oral toxicity/pathogenicity study in rats. (Btk Tox Batch PPQ 2843): Lab Project No. 89123. Unpublished study prepared by Novo Nordisk A/S, Enzyme Tox Lab. 43 p. MRID 41653903

Harde T. 1990b. *Bacillus thuringiensis* var. *kurstaki* Acute oral toxicity/pathogenicity study in rats given Btk Tox Batch PPQ 2843. 3(NB 75): Lab Project No. NOVO/REBF/VOL5. Unpublished study prepared by Enzyme Toxicology Laboratory. 43 p. MRID 41657004

Harde T. 1991. *Bacillus thuringiensis* var. Israelensis: Acute oral toxicity/pathogenicity study in rats given Bti Tox Batch PPQ 3044. (NB 31): Lab Project No. 90055: NOVO/SFCRERE/VOL5. Unpublished study prepared by Novo Nordisk A/S. 47 p. MRID 41980102

Hargrove J. 1990a. Manufacturing process description and discussion of the formation of unintentional ingredients for the production of certain biological insecticide: Lab Project No. 011990-E. Unpublished study prepared by Sandoz Crop Protection Corp. 34 p. MRID 41490801

Hargrove J. 1990b. Manufacturing process description and discussion of the formation of unintentional ingredients for the production of Teknar Biological Insecticide: Lab Project No. 011990-C. Unpublished study prepared by Sandoz Crop Protection Corp. 34 p. MRID 41490802

Hargrove J. 1990c. Manufacturing Process description and discussion of formation of the formation of unintentional ingredients for the production of Teknar HPD: Lab Project No. 011990-B. Unpublished study prepared by Sandoz Crop Protection Corp. 34 p. MRID 41490803

Haverty MI. 1982. Sensitivity of selected nontarget insects to the carrier of Dipel 4L in the laboratory. Environ Entomol. 11:337-338

Helgason E; Okstad OA; Caugant DA; Johansen HA; Fouet A; Mock M; Hegna I; Kolsto. 2000. *Bacillus anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis* – One species on the basis of genetic evidence. Appl Environ Microbiol. (6):2627-26230

Hellmich RL; Siegfried BD; Sears MK; Stanley-Horn DE; Daniels MJ; Mattila HR; Spencer T; Bidne KG; Lewis LC. 2001. Monarch larvae sensitivity to *Bacillus thuringiensis*- purified proteins and pollen. Proc Natl Acad Sci USA. 98(21):11925-11930

Hendriksen NB; Hansen BM. 1998. Phylogenetic relations of *Bacillus thuringiensis*: Implications for risks associated to its use as a microbiological pest control agent. IOBC Bull. 21:5-8

Herms CP; Mccullough DG; Baue LS; Haack RA; Miller DL; Dubois NR. 1997. Susceptibility of the endangered Karner blue butterfly (Lepidoptera: lycaenidae) to *Bacillus thuringiensis* var. *kurstaki* used for gypsy moth suppression in Michigan. Great Lakes Entomol. 30:125-141

Hernandez E; Ramisse F; Ducoureau JP; Cruel T; Cavallo JD. 1998. *Bacillus thuringiensis* subsp. *konkukian* (Serotype H34) superinfection: Case report and experimental evidence of pathogenicity in immunosuppressed mice. J Clinl Microbiol. 36(7):2138-2139

Hernandez E; Ramisse F; Cruel T; Vagueresse R; Cavallo JD. 1999. *Bacillus thuringiensis* serotype H34 isolated from human and insecticidal strains serotypes 3a3b and H14 can lead to death of immunocompetent mice after pulmonary infection. FEMS Immunol Med Microbiol. 24:43-47

Hernandez E; Ramisse F; Gros P; Cavallo JD. 2000. Super-infection by *Bacillus thuringiensis* H34 or 3a3b can lead to death in mice infected with influenza A virus. FEMS Immunology Med Microbiol. 29:177-181

Hickle LA; Fitch WL. 1990. Analytical Chemistry of *Bacillus thuringiensis*. ACS Symposium Series 432, American CheChemical Society, Washington, DC, 148 pp.

Hofte H; Whiteley HR. 1989. Insecticidal Crystal Proteins of *Bacillus thuringiensis*. Microbiol Revs. 53:242-255

Holbert M. 1990. Acute intravenous toxicity/pathogenicity study in rats with a microbial pest control agent.(MCPA) consisting of viable microbes and non-viable organisms: Lab Project No. 6892-90. Unpublished study prepared by Stillmeadow, Inc. 19 p. MRID 41751107

Holbert M. 1991. Foray 76B: Acute Inhalation Toxicity Study in Rats with MPCA. EPA Guidelines No. 81-3. Sept. 26, 1991, Stillmeadow, Inc. Laboratory Study No. 8163-91

Honda T; Shiba A; Seo S; Yamamoto J; Matsuyama J; Miwatani T. 1991. Identity of hemolysins produced by *Bacillus thuringiensis* and *Bacillus cereus*. FEMS Microbiol Lett Fed Eur Microbiol Soc. 79:205-210

Hossack D. 1990a. Acute oral toxicity and infectivity/pathogenicity study of CGA-237218. (*Bacillus thuringiensis* var. *aizawai*) in rats: Lab Project No. CBG 517-1. Unpublished study prepared by Huntingdon Research Centre, Ltd. 35 p. MRID 42006502

Hossack D. 1990b. Acute Pulmonary toxicity and infectivity/pathogenicity study of CGA-237218. (*Bacillus thuringiensis* var. *aizawai* in rats: Lab Project No. CBG 517-2. Unpublished study prepared by Huntingdon Research Centre, Ltd. 40 p. MRID 42006503

Hoxter K; Smith G. 1991. A dietary pathogenicity and toxicity study with ladybird beetles: Lab Project No. 297-102B. Unpublished study prepared by Wildlife International Ltd. 17 p. MRID 41751112

Hoxter K; Smith G. 1991. *Bacillus thuringiensis* var. *israelensis*: Strain NB31, Tox Batch PPQ 3044: A dietary pathogenicity and toxicity study with the honey bee: Lab Project No. 254-120. Unpublished study prepared by Wildlife International Ltd. 20 p. MRID 41842711

Hoxter K; Thompson M; Jaber M. 1990a. BTK Toxbatch NB 75 Batch No. PPQ 2843: A dietary pathogenicity and toxicity study with the green lacewing larvae in support of registration of Biobit Flowable Concentrate: Lab Project No. 254/117. Unpublished study prepared by Wildlife International. 16p. MRID 41657011

Hoxter K; Smith G; Jaber M. 1990b. A Dietary pathogenicity and toxicity study with the parasitic Hymenopteran Uga menoni: Lab Project No. 297-103. Unpublished study prepared by Wildlife International Ltd. 16 p. MRID 41751110

Idris AB; Grafius E. 1993. Differential toxicity of pesticides to *Diadegma insulare* (Hymenoptera: Ichneumonidae) and its host, the diamondback moth (Lepidoptera: Plutellidae). J Econ Entomol. 86(2):529-536

Ignoffo CM. 1973. Effects of entomopathogens on vertebrates. Ann NY Acad Sci. 217:141-164

Innes DGL; Bendell JF. 1989. The effects on small-mammal populations of aerial applications of *Bacillus thuringiensis*, fenitrothion, and Matacil(R) used against jack pine budworm in Ontario. Can J Zool. 67: 318-1323

Iriarte J; Bel Y; Ferrandis MD; Andrew R; Murillo J; Ferre J; Caballero P. 1998. Environmental distribution and diversity of *Bacillus thuringiensis* in Spain. Syst Appl Microbiol. 21:97-106

Isaacson J. 1991a. Analysis of â-exotoxin (thuringiensis) content of five lots of VectoBac TP by housefly bioassay: Lab Project No. 910/9011. Unpublished study prepared by Abbott Laboratories. 12 p. MRID 41880001

Isaacson J. 1991b. Analysis of â-exotoxin (thuringiensis) content of five lots of DiPel TP by housefly bioassay: Lab Project No. 910-9010. Unpublished study prepared by Abbott Labs. 12 p. MRID 41883801

Jackson SG; Goodbrand RB; Ahmed R; Kasatiya S. 1995. *Bacillus cereus* and *Bacillus thuringiensis* isolated in a gastroenteritis outbreak investigation. Lett Appl Microbiol. 21:103-105

James RR; Miller JC; Lighthart B. 1993. *Bacillus thuringiensis* var. *kurstaki* affects a beneficial insect, the cinnabar moth (Lepidoptera: arctiidae). J Econ Entomol. 86:334-339

Jayanthi P DK; Padmavathamma K. 1997. Laboratory evaluation of toxicity of *Bacillus thuringiensis* subsp. *kurstaki* to larvae of mulberry silkworm, *Bombyx mori*. J Entomol Res (New Delhi). 21(1):45-50

Jenkins JL; Lee MK; Valaitis AP; Curtiss A; Dean DH. 2000. Bivalent sequential binding model of a *Bacillus thuringiensis* toxin to gypsy moth aminopeptidase N receptor. J Biol Chem. 275(19):14423-14431

Jensen B; Rugh S; Overholt J. 1990a. Product analysis data: Product Identity and manufacturing information in support of reregistration of Biobit Wettable Powder: Lab Project Nos. 90- 0120: 90-0090: 90-0101. Unpublished study prepared by Novo-Nordisk A/S & Novo Laboratories, Inc. 303 p. MRID 41459401

Jensen B; Rugh S; Overholt J. 1990b. Product analysis data: product identity and manufacturing information in support of reregistration of Biobit Flowable Concentrate: Lab Project No. F-890043: HG/TONI/JMO: F-882320. Unpublished study prepared by Novo-Nordisk A/S and Novo Laboratories. MRID 41459402

Jensen B; Rugh S; Overholt J. 1990c. Product analysis data: Product identity and manufacturing information in support of reregistration of Skeetal Flowable Concentrate: Lab Project No. 90006: AF/265/1/GB: F-893084. Unpublished study prepared by Novo-Nordisk A/S & Novo Laboratories, Inc. MRID 41459403

Jensen B; Rugh S; Overholt J. 1990d. Product Chemistry Data: Physical and Chemical Properties in Support of Reregistration of Biobit Wettable Powder. Unpublished study prepared by Novo Nordisk A/S & Novo Laboratories, Inc. 7 p. MRID 41503901

Jensen B; Rugh S; Overholt J. 1990e. Product chemistry data: Physical and chemical properties in support of reregistration of Biobit Flowable Concentrate. Unpublished study prepared by Novo Nordisk A/S & Novo Laboratories. 8 p. MRID 41503902

Jensen B; Rugh S; Overholt J. 1990f. Product chemistry data: Physical and chemical properties in support of reregistration of Skeetal Flowable Concentrate. Unpublished study prepared by Novo Nordisk A/S & Novo Laboratories, Inc. 8 p. MRID 41503903

Jensen B; Sorensen E; Rugh S; et al. 1991g. Product analysis data: sample analysis and analytical methods: Skeetal Flowable Concentrate: Lab Project No. NOVO/SFCRERE/ VOL3. Unpublished study prepared by Novo Nordisk A/S & Novo Nordisk Bioindustrials Inc. 41 p. MRID 41980101

Jensen GB; Larsen P; Jacobsen BL et al. 2002. Isolation and characterization of bacillus cereus-like bacteria from faecal samples from greenhouse workers WHO are using *Bacillus thuringiensis*-based insecticides. Int Arch Occup Environ Hlth. 75(3):91-96

Johnson KS; Scriber JM; Nitao JK; Smitley DR. 1995. Toxicity of *Bacillus thuringiensis* var. *kurstaki* to three nontarget lepidoptera in field studies. Environ Entomol. 24(2):288-297

Jolivet P. 1999. La Menace des Insectes: Un Nouveau Casse-Tet pour les Entomoligistes: Les Bacteries des Insectes attaquent-elles aussi l'Homme. L'Entomologiste. 55:73-78

Jyoti JL; Brewer GJ. 1999. Honey bees (Hymenoptera: apidae) as vectors of *Bacillus thuringiensis* for control of banded sunflower moth (Lepidoptera: tortricidae). Environ Entomol. 28:1172-1176

Kiehr B. 1991a. Acute Dermal toxicity study in rabbits with the end product Foray 75B, batch BBN 7001. Enzyme Toxicology Laboratory; February 6, 1991; 15 p. Sponsored by Novo Nordkisk A/S, Bagsvaerd, Denmark

Kiehr B. 1991b. Eye irritation study in rabbits with the end product Foray 75b, batch BBN 7001. Enzyme Toxicology Laboratory; February 6, 1991; 19 p. Sponsored by Novo Nordkisk A/S, Bagsvaerd, Denmark

Kirkland R. 1991. The Effect of *Bacillus thuringiensis*, ABG-6305 technical powder, on the honeybee. (*Apis mellifera* L.): Lab Project No. CAR 196-90. Unpublished study prepared by California Agricultural Research, Inc. 52 p. MRID 41974808

Knoll H. 1990a. *Bacillus thuringiensis kurstaki*: generic and manufacturing use product data. Unpublished study prepared by Knoll Bioproducts Company, Inc. 14 p. MRID 42015901

Knoll H. 1990b. Generic acute oral and acute pulmonary toxicity and pathogenicity data 152A-10 and 152A-12, and intravenous toxicity/pathogenicity data 152A-13. Unpublished study prepared Knoll Bioproducts Co., Inc. 19 p. MRID 42016001

Koskella J; Stotzky G. 1997. Microbial utilization of free and clay-bound insecticidal toxins from *Bacillus thuringiensis* and their retention of insecticidal activity after incubation with microbes. Appl Environ Microbiol. 63(9):3561-3568

Kouassi KC; Lorenzetti F; Guertin C; Cabana J; Mauffette Y. 2001. Variation in the susceptibility of the forest tent caterpillar (Lepidoptera: lasiocampidae) to *Bacillus thuringiensis* variety *kurstaki* HD-1: Effect of the host plant. J Econ Entomol. 94(5):1135-1141

Kreutzweiser DP; Capell SS. 1992. A simple stream-side test system for determining acute lethal and behavioral effects of pesticides on aquatic insects. Environ Toxicol Chem. 11:993-999

Kreutzweiser DP; Capell SS. 1996. Palatability of leaf material contaminated with *Bacillus thuringiensis* var. *kurstaki*, to *Hydatophylax argus*, a detritivorous aquatic insect. Bull Environ Contam Toxicol. 56:80-84

Kreutzweiser DP; Holmes SB; Capell SS; Eichenberg DC. 1992. Lethal and sublethal effects of *Bacillus thuringiensis* var. *kurstaki* on aquatic insects in laboratory bioassays and outdoor steam channels. Bull Environ Contam Toxicol. 49:252-257

Kreutzweiser DP; Capell SS; Thomas DR. 1994. Aquatic insect responses to *Bacillus thuringiensis* var. *kurstaki* in a forest stream. Can J For Res. 24(10):2041-2049

Kreutzweiser DP; Gringorten JL; Thoomas DR; Butcher JT. 1996. Functional effects of the bacterial insecticide *Bacillus thuringiensis* var. *kurstaki* on aquatic microbial communities. Ecotoxicol Environ Saf. 33:271-280

Krieg A; Herfs W. 1963. The effects of *Bacillus thuringiensis* on honey bees. Entomol Exp Appl. 6:1-9

Kuhn J. 1991. Acute oral toxicity study in rats: U.S. EPA Guidelines No. 81-1. Stillmeadow, Inc.; June 28, 1991; 12 p. Sponsored by Novo Nordisk A/S, Bagsvaerd, Denmark

Kuhn J. 1998a. Dipel ES (ABG-6158) Acute Dermal Toxicity Study in Rats. Lab Project Number: 4434-98. Unpublished study prepared by Stillmeadow, Inc. 17 p. MRID 44791609

Kuhn J. 1998b. Dipel ES (ABG-6158) Acute Oral Toxicity Study in Rats. Lab Project Number: 4433-98. Unpublished study prepared by Stillmeadow, Inc. 15 p. MRID 44791608

Kuhn J. 1999a. Dipel ES (ABG-6158) Primary Dermal Irritation Study in Rats. Lab Project Number: 4437-98. Unpublished study prepared by Stillmeadow, Inc. 18 p. MRID 44791612

Kuhn J. 1999b. Dipel ES (ABG-6158) Primary Eye Irritation Study in Rats. Lab Project Number: 4436-98. Unpublished study prepared by Stillmeadow, Inc. 22 p. MRID 44791611

Laferriere M; Bastille A; Nadeau A. 1987. Immunologic Study of the Components of the Biological Insecticide *Bacillus thuringiensis* var *kurstaki*. Public Health Administration, Grand-Portage Regional Hospital Center, Quebec.

Lankas GR; Hogan GK; Fasanella J; et al. 1981a. A Single Oral Dose Toxicity/Infectivity Study of Thuricide 32 B in Rats: Project No. 80-2523; Report No. T-1-2/23/81. (Unpublished study received Mar 8, 1982 under 11273-2; d by Bio/dynamics, Inc., submitted by Sandoz, Inc.--Crop Protection, San Diego,Calif.; CDL:246967-A). MRID 00096527

Lankas G; McCormack R; Hogan G; et al. 1981b. Single Oral Dose Toxicity/Infectivity Study of Thuricide 32B in the Rat: Project No. 80-2523; Report No. T-1-2/23/81. Final rept. (Unpublished study received Aug 9, 1982 under 11273-2; prepared in cooperation with Bio/dynamics, Inc., submitted by Sandoz, Inc., Crop Protection, San Diego, CA; CDL:248007-E). MRID 00109492

Lankas G; McCormack R; Hogan G; et al. 1981c. Acute Dermal Toxicity/Infectivity Study of Thuricide 32B in the Rat: Project No. 80-2531; Report No. T-1-3/11/81. Final rept. (Unpublished study received Aug 4, 1982 under 11273-2; prepared in cooperation with Bio/dynamics, Inc., submitted by Sandoz, Inc., Crop Protection, San Diego, CA; CDL:248007-F). MRID 00109493

Lattin A. 1990a. CGA-237218 Technical (GC-91): An Avian Oral Pathogenicity and Toxicity Study in the Bobwhite: Lab Project Number 108-308. Unpublished study prepared by Wildlife International Ltd. 21 p. (GC-91): An Avian Oral Pathogenicity and Toxicity Study in the Bobwhite: Lab Project Number 108-308. Unpublished study prepared by Wildlife International Ltd. 21 p. MRID 41994313

Lattin A. 1990b. CGA-237218 Technical (GC-91): An Avian Oral Pathogenicity and Toxicity Study in the Mallard: Lab Project Number 108-309. Unpublished study prepared by Wildlife International Ltd. 22 p. (GC-91): An Avian Oral Pathogenicity and Toxicity Study in the Mallard: Lab Project Number 108-309. Unpublished study prepared by Wildlife International Ltd. 22 p. MRID 41994314

Lattin A; Grimes J; Hoxter K; et al. 1990a. Vectobac Technical Material (*Bacillus thuringiensis* var *israelensis*): An Avian Oral Toxicity and Pathogenicity Study in the Mallard: Project No. 161-115. Unpublished study prepared by Wildlife International Ltd. 24 p. MRID 41439005

Lattin A; Grimes J; Hoxter K; et al. 1990a. Dipel Technical Material (*Bacillus thuringiensis* var *kurstaki*): An Avian Oral Toxicity and Pathogenicity Study in the Bobwhite. Lab Project Number: 161-112. Unpublished study prepared by Wildlife International Ltd. 25 p. MRID 41443404

Lattin A; Hoxter K; Smith G. 1990b. Vectobac Technical Material (*Bacillus thuringiensis* var *israelensis*): An Avian Oral Toxicity and Pathogenicity Study in the Bobwhite: Project No. 161-114. Unpublished study prepared by Wildlife International Ltd. 29 p. MRID 41439006

Lattin A; Hoxter K; Driscoll C; et al. 1990b. Dipel Technical Material (*Bacillus thuringiensis* var *kurstaki*): An Avian Oral Toxicity and Pathogenicity Study in the Mallard. Lab Project No: 161-113. Unpublished study prepared by Wildlife International Ltd. 28 p. MRID 41443403

Lattin A; Hoxter K; Driscoll C; et al. 1990c. Dipel Technical Material (*Bacillus thuringiensis* var *kurstaki*): An Avian Oral Toxicity and Pathogenicity Study in the Mallard: Lab Project No: 161-113. Unpublished study prepared by Wildlife International Ltd. 28 p. MRID 41443403

Lattin A; Hoxter K; Jabber M. 1990c. An Avian Oral Pathogenicity and Toxicity Study in the Mallard: Biobit Wettable Powder (Batch PPQ 2843). Lab Project Number: 254-113. Unpublished study prepared by Wildlife International Ltd. 27 p. MRID 41653906

Lattin A; Grimes J; Hoxter K; et al. 1990d. Dipel Technical Material (*Bacillus thuringiensis* var *kurstaki*): An Avian Oral Toxicity and Pathogenicity Study in the Bobwhite: Lab Project Number: 161-112. Unpublished study prepared by Wildlife Intertional Ltd. 25 p. MRID 41443404

Lattin A; Hoxter K; Jaber M. 1990e. BTK Toxbatch NB75 Batch No. PPQ 2843: An Avian Oral Pathogenicity and Toxicity Study in Bobwhite in Support of Registration of Biobit Flowable Concentrate: Lab Project Number: 254/114. Unpublished study prepared by Wildlife International Ltd. 27 p. MRID 41657007

Lattin A; Hoxter K; Jaber M. 1990f. BTK Toxbatch NB75 Batch No. PPQ 2843: An Avian Oral Pathogenicity and Toxicity Study in the Mallard in Support of Registration of Biobit Flowable Concentrate: Lab Project Number: 254/113. Unpublished study prepared by Wildlife International Ltd. 27 p. MRID 41657008

Lattin A; Hoxter K; Jabber M. 1990g. An Avian Oral Pathogenicity and Toxicity Study in the Mallard: Biobit Wettable Powder (Batch PPQ 2843): Lab Project Number: 254-113. Unpublished study prepared by Wildlife International Ltd. 27 p. MRID 41653906

Lee BM; Scott GI. 1989. Acute toxicity of temephos, fenoxycarb, diflubenzuron, and methoprene and *Bacillus thuringiensis* var. *israelensis* to the mummichog (*Fundulus heterocilitus*). Bull Environ Contam Toxiciol. 43:827-832

Lee IH; Je YH; Chang JH; Roh JY; Oh HW; Lee SG; Shin SC; Boo KS. 2001. Isolation and characterization of a *Bacillus thuringiensis* ssp. *kurstaki* strain toxic to *Spodoptera exigua* and *Culex pipiens*. Cur Microbiol. 43(4):284-287

Leeper L. 1999a. Dipel ES (ABG-6158) Acute Inhalation Toxicity Study in Rats. Lab Project Number: 4435-98. Unpublished study prepared by Stillmeadow, Inc. 22 p. MRID. 44791610

Leeper L. 1999b. DiPel Technical Powder (ABG-6302) Acute Inhalation Toxicity Study in Rats. Lab Project Number: 4441-98. Unpublished study prepared by Stillmeadow, Inc. 22 p. MRID 44791606

Leong KFH; Cano RJ; Kubinski AM. 1980. Factors affecting *Bacillus thuringiensis* total field persistence. Environ Entomol. 9:593-599

Leong KLH; Yoshimura MA; Kaya HK. 1992. Low susceptibility of overwintering monarch butterflies to *Bacillus thruingiensis berline*. Pan Pacific Entomol. 68:66-68

Li MS; Je YH; Lee IH; Chang JH; Roh JY; Kim HS; Oh HW; Boo KS. 2002. Isolation and characterization of a strain of *Bacillus thuringiensis* ssp. *kurstaki* containing a New delta-endotoxin gene. Cur Microbiol. 45(4):299-302

Liu M; Cai QX; Liu HZ; Zhang BH; Yan JP; Yuan ZM. 2002. Chitinolytic activities in *Bacillus thuringiensis* and their synergistic effects on larvicidal activity. J Appl Microbiol. 93(3):374-379

Maczuga SA; Mierzejewski KJ. 1995. Droplet size and density effects of *Bacillus thuringiensis kurstaki* on gypsy moth (Lepidoptera: lymantriidae) larvae. J Econ Entomol. 88:1376-1379

Maeda M; Mizuki E; Nakamura Y; Hatano T; Ohba M. 2000. Recovery of *Bacillus thuringiensis* from marine sediments of Japan. Cur Microbiol. 40(6):418-422

Maeda M; Mizuki E; Hara M; Tanaka R; Akao T; Yamashita S; Ohba M. 2001. Isolation of *Bacillus thuringiensis* from intertidal brackish sediments in mangroves. Microbiol Res. 156(2):195-198

Martin P AW; Baya AM; Navarro R; Evans J. 1997. Sublethal effects of *Bacillus thuringiensis* on koi carp cyprinus carpio. 97th General Meeting of the American Society for Microbiology, Miami Beach, Florida, Usa, May 4-8, 1997. Abstr Gen Mtg Am Soc Microbiol. 97:388

Martinez C; Caballero P. 2002. Contents of cry genes and insecticidal toxicity of *Bacillus thuringiensis* strains from terrestrial and aquatic habitats. J Appl Microbiol. 92(4):745-52

Masse A; Van Frankenhuyszen K; Dedes J. 2000. Susceptibility and vulnerability of third-instar larvae of the spruce budworm (Lepidoptera: tortricidae) to *Bacillus thuringiensis* subsp. *kurstaki*. Can Entomol. 132:573-580

Mayer FL; Ellersieck MR. 1986. Manual of acute toxicity: interpretation and data base of 410 chemicals and 66 species of freshwater animals. U.S. Department of the Interior, Fish and Wildlife Service, Resource Publication 160, Washington, DC. Summarized in USDA 1995

McClintock JT; Schaffer CR; Kough JL; Sjoblad RD. 1995a. Relevant Taxonomic Considerations for Regulation of *Bacillus thuringiensis*-Based Pesticides by the U.S. Environmental Protection Agency. In T-Y Feng, et al. (eds.), "*Bacilus thuringiensis* Biotechnology and Environmental Benefits.", Vol. I, 313-325

McClintock JT; Schaffer CR; Sjoblad RD. 1995b. A Comparative Review of the Mammalian Toxicity of *Bacillus thuringiensis*- Based Pesticides. Pestic Sci. 45:95-105

McDonald P; Scott DG. 1991. Foray 48B, FC: Acute inhalation toxicity study in rats (limit test). Inveresk Research International, Ltd.; August 10, 1991; 36 p. Sponsored by Novo Nordisk A/S, Bagsvaerd, Denmark

Meadows MP. 1993. *Bacillus thuringiensis* in the environment: Ecology and risk assessment. In: Entwistle PF, Cory JS, Bailey MJ, & Higgs S ed. *Bacillus thuringiensis*, an environmental biopesticide: Theory and practice. Chichester, NY: Wiley & Sons. p. 193-220

Meher SM; Bodhankar SL; Arunkumar; Dhuley JN; Khodape DJ; Naik SR. 2002. Toxicity studies of microbial insecticide *Bacillus thuringiensis* var. *kenyae* in rats, rabbits, and fish. Int J Toxicol. 21(2):99-105

Menon AS; De Mestral S. 1985. Survival of *Bacillus-thuringiensis*-var-*kurstaki* in waters. Water, Air, Soil Pollut. 25:265-274

Meretoja T et al. 1977. Mutagenicity of *Bacillus thuringiensis* exotoxin: 1. Mammalian tests. Hereditus. 85:105-112

Meshram PB; Bisaria AK; Kalia S. 1997. Efficacy of bioasp and biolep a microbial insecticide against teak skeletonizer eutectona machaeralis walk. Indian Forester. 123(12):1202-1204

Mignot T; Mock M; Robichon D; Landier A; Lereclus D; Fouet A. 2001. The incompatibility between the plcr- and atxa-controlled regulons may have selected a nonsense mutation in *Bacillus anthracis*. Mol Microbiol. 42(5):1189-1198

Miller JC. 1990a. Effects of a microbial insecticide, *Bacillus thuringiensis kurstaki*, on nontarget lepidoptera in a spruce budworm-infested forest. J Res Lepid. 29:267-276

Miller JC. 1990b. Field assessment of the effects of a microbial pest control agent on nontarget lepidoptera. American Entomologist. 36:135-139

Milner RJ. 1994. History of *Bacillus thuringiensis*. Agriculture Ecosystems and Environment. 49:9-13

Moar WJ; Pusztai-Carey M; Mack TP. 1995. Toxicity of purified proteins and the HD-1 strain from *Bacillus thuringiensis* against lesser cornstalk borer (Lepidoptera: pyralidae). J Econ Entomol. 88: 606-609

Mohaghegh J; Clercq P de; Tirry L. 2000. Toxicity of selected insecticides to the spined soldier bug, *Podisus maculiventris* (Heteroptera: pentatomidae). Biocontrol Sci Technol. 10: 33-40

Mohan M; Gujar GT. 2000. Susceptibility pattern and development of resistance in the diamondback moth, *Plutella xylostella*, to *Bacillus thuringiensis berl* var *kurstaki* in India. Pest Manag Sci. 56:189-194

Mott M; Smitley D. 2000. Impact of *Bacillus thuringiensis* application on entomophaga maimaiga (Entomophthorales: entomophthoraceae) and ldnpv-induced mortality of gypsy moth (Lepidoptera: lymantriidae). Environ Entomol. 29:1312-1322

Murakami T; Hiraoka T; Matsumoto T; Katagiri S; Shinagawa K; Suzuki M. 1993. Analysis of common antigen of flagella in *Bacillus cereus* and *Bacillus thuringiensis*. FEMS Microbiol Lett. 107:79-184

Nagy LR; Smith KG. 1997. Effects of insecticide-induced reduction in lepidopteran larvae on reproductive success of hooded warblers. The Auk. 11:619-627

Navon A. 1993. Control of lepidopteran pests with *Bacillus thuringiensis*. *In: Bacillus thuringiensis*, an Environmental Biopesticide: Theory and Practice, Entwistle, P. F., et al. (Eds). John Wiley and Sons, Inc, New York, NY. p.125-146.

Navon A; Keren S; Levski S; Grinstein A; Riven Y. 1997. Granular feeding baits based on *Bacillus thuringiensis* products for the control of lepidopterous pests. Phytoparasitica. 25(Suppl.):101S-110S.

Nelson R. 1990. The effect of the microbial pest control agent *Bacillus thuringensis* subsp. *kurstaki* on the predatory mite *Metaseiulus occidentalis* (Nesbit) and their host prey the twospotted spider mite *Tetranychus urticae*. (Koch): Lab Project No:90.020: Protocol No. I-PSI-NTO-PM-90. Unpublished study prepared by Plant Sciences, Inc. 39 p. MRID 41443410

Nelson R. 1991a. The effect of *Bacillus thuringiensis*, ABG-6305 technical powder, on the predatory mite (Nesbit) and their host prey the twospotted spider mite *Tetranychus urticae*. (Koch): Lab Project Number: 91.042. Unpublished study prepared by Plant Sciences, Inc. 38 p. MRID 41974809

Nelson R. 1991b. The Effects of *Bacillus thuringiensis*, ABG-6305 technical powder, on the common green lacewing, *Chrysoperla Carna* (Stephens): Lab Project Number: 91.043. Unpublished study prepared by Plant Sciences, Inc. 30 p. MRID 42245301

Newton P. 1999. A 4-week inhalation toxicity study of dimilin technical in rats. Lab Project Number: 399-205. Unpublished study prepared by MPI Research, Inc. 357 p. MRID No. 44950601

Nielsen-LeRoux C; Hansen BM; Henriksen NB. 1998. Safety of *Bacillus thuringiensis*. IOBC Bull. 21: 269-272

Noble MA; Riben PD; Cook GJ. 1992. Microbiological and epidemiological surveillance programme to monitor the health effects of Foray 48B BTK spray. Vancouver, Canada, Ministry of Forests of the Province of British Columbia. p. 1-63

Norton ML; Bendell JF; Bendell-Young LI; Leblanc CW. 2001. Secondary effects of the pesticide *Bacillus thuringiensis kurstaki* on chicks of spruce grouse (*Dendragapus canadensis*). Arch Environ Contam Toxicol. 41(3):369-373

Notermans S; Batt CA. 1998. A risk assessment approach for food-borne *Bacillus cereus* and its toxins. J Appl Environ Microbiol Symp. 84(suppl): 51S-61S

Novo Nordisk Bioindustrials Inc. 1991. Submission of additional data regarding unreasonable adverse effects of Foray 48B on humans for section 6(a).)(2) Requirements. Transmittal of 1 study. MRID 42027100

O'Leary P. 1990. Effect of *Bacillus thuringiensis* subsp. *kurstaki* on the common green lacewing, *Chrysoperla carnea* (Stephens): Lab Project Number: LR90-406. Unpublished study prepared by PanAgricultural Laboratories. 41 p. MRID 41443411

Oh K-S; Oh B-Y; Park S-S; Lee J-K. 1998. Improvement of *Bacillus thuringiensis* wettable powder to enhance adherence. RDA J Crop Protect. 40(1):57-62.

Oregon Health Services. 2003. Questions and answers about gypsy moth spraying and your health. Available at: http://www.dhs.state.or.us/publichealth/pesticide/btkfacts.cfm

Oshodi R; Macnaughtan R. 1990a. BTK preparation: Acute inhalation toxicity study in rats in support of registration of Biobit flowable concentrate: Lab Project Number: NOVO/REBF/VOL6. Unpublished study prepared by Inveresk Research International. 44 p. MRID 41657005

Oshodi R; Macnaughtan R. 1990b. BTK preparation: Acute inhalation toxicity study in rats: 48B Foray: Lab Project No. IRI 644583: NOVO/REF/VOL6. Unpublished study prepared by Inveresk Research International. 44 p. MRID 41917001

Oshodi R; Macnaughtan R. 1990c. BTK preparation: Acute inhalation toxicity study in rats. Lab Project Number: 644583: NOVO/REBW/VOL6. Unpublished study prepared by Inveresk Research International. 44 p. MRID 41917601

Oshodi R; Robb D. 1990. BTi preparation: acute inhalation toxicity study in rats: Skeetal Flowable concentrate: Lab Project Number: 650314. Unpublished study prepared by Inveresk Research International. 49 p. MRID 41980103

Otvos I.S; Vanderveen S. 1993. Environmental report and current status of *Bacillus thuringiensis* var. *kurstaki*. use for control of forest and agricultural insect pests. Province of British Columbia Forestry Canada report. 81 pp. (Cited in USDA 1995)

Palmer S; Beavers J. 1993. Xentari technical powder (ABG-6305): A dietary pathogenicity and toxicity study with the ladybird beetle (*Hippodamia convergens*). Final Report: Lab Project Number: 161-126A. Unpublished study prepared by Wildlife International Ltd. 36 p. MRID 42942101

Pang ASD; Gringorten JL. 1998. Degradation of *Bacillus thuringiensis* delta-endotoxin in host insect gut juice. Fems Micro Biol Lett. 167: 281-285

Paulus R; Roembke J; Ruf A; Beck L. 1999. A comparison of the litterbag-, minicontainer- and bait-lamina-methods in an ecotoxicological field experiment with diflubenzuron and Btk. Pedobiologia. 43(2):120-133

Peacock JW; Schweitzer DF; Carter JL; Dubois NR. 1998. Laboratory assessment of the effects of *Bacillus thuringiensis* on native lepidoptera. Environ Entomol. 27: 450-457

Pearce M; Habbick B; Williams J; Eastman M; Newman M. 2002. The effects of aerial spraying with *Bacillus thuringiensis kurstaki* on children with asthma. Can J Public Health. 93(1):21-25

Perez CJ; Shelton AM; Roush RT. 1997a. Managing diamondback moth (Lepidoptera: plutellidae) resistance to foliar applications of *Bacillus thuringiensis*:Ttesting strategies in field cages. J Econ Entomol. 90: 1462-1470

Perez CJ; Tang JD; Shelton AM. 1997b. Comparison of leaf-dip and diet bioassays for monitoring *Bacillus thuringiensis* resistance in field populations of diamondback moth (Lepidoptera: plutellidae). J Econ Entomol. 90: 94-101

Perneger TV. 1998. What's wrong with Bonferroni adjustments. BMJ. 316(7139):1236-1238

Peter S; Boon B. 1990a. Registration Standard No. 0247: *Bacillus thuringiensis* var. israelensis: Bactomos primary powder: Product analysis data: Lab Project Number: BT:RS: 56637/17/90. Unpublished study prepared by Solvay & Cie. 16 p. MRID 41429703

Peter S; Boon B. 1990b. Registration Standard No. 0247: *Bacillus thuringiensis* var. israelensis: Bactomos primary powder: physical and chemical properties: Lab Project Number: BT:RS: 566/37/18/90. Unpublished study prepared by Solvay & Cie. 5 p. MRID 41429704

Peter S; Boon B; Charmoille L. 1990. Registration Standard No. 0247: *Bacillus thuringiensis* var. israelensis: Bactomos primary powder: Product identity and disclosure of ingredients: Lab

Project Number: 56637/10/90: BT:RS. Unpublished study prepared by Solvay & Cie. 59 p. MRID 41429701

Peter J; Boon B; Malcorps C. 1990. Registration Standard No. 0247: *Bacillus thuringiensis* var. israelensis: Bactomos primary powder: description of manufacturing process: Lab Project Number: BT:RS: 56637/16/90. Unpublished study prepared by Solvay & Cie. 89 p. MRID 41429702

Petrie K; Thomas M; Broadbent E. 2003. Symptom complaints following aerial spraying with biological insecticide Foray 48B. N Z Med J. 116: 1170-1177. Available at: http://www.nzma.org.nz/journal/116-1170/

Raps A; Kehr J; Gugerli P; Moar WJ; Bigler F; Hilbeck A. 2001. Immunological analysis of pH sap of *Bacillus thuringiensis* corn and of the nontarget herbivore *Rhopalosiphum padi* (Homoptera: aphididae) for the presence of cry1ab. Mol Ecol. 10: 525-533

Rastall K; Kondo V; Strazanac JS; Butler L. 2003. Lethal effects of biological insecticide applications on nontarget lepidopterans in two Appalachian forests. Environ Entomol. 32(6): 1364-1369.

Rausina G. 1949. Report to United States Department of Agriculture, Agricultural Research Service, Agricultural Environmental Quality Institute: Results of four-day static fish toxicity studies: Rainbow trout and Bluegills: IBT No. A-1958. MRID No. 59735

Reardon RC; Wagner DL. 1995. Impact of *Bacillus thuringiensis* on nontarget lepidopteran species in broad-leaved forests. American Chemical Society, Washington, DC. p. 284-292

Reardon R; Dubois N; McLane W. 1994. *Bacillus thuringiensis* for managing gypsy moth: A review. National Center of Forest Health Management, USDA Forest Service. FHM-NC-01-94, January 1994

Regniere J; Cooke B. 1998. Validation of a process-oriented model of *Bacillus thuringiensis* variety *kurstaki* efficacy against spruce budworm (Lepidoptera: tortricidae). Environ Entomol. 27: 801-811

Ren Z; Ma E; Guo Y. 2002. Chromosome aberration assays for the study of cyclophosphamide and *Bacillus thuringiensis* in oxya chinensis (Orthoptera: acrididae). Mutat Res. 520(1-2):141-150

Richardson JS; Perrin CJ. 1994. Effects of bacterial insecticide *Bacillus thuringiensis* var. *kurstaki* (Btk) on a stream benthic community. Canad J Fish Aquatic Sci. 51: 1037-1045

Robacker DC; Martinez AJ; Garcia JA; Diaz M; Romero C. 1996. Toxicity of *Bacillus thuringiensis* to Mexican fruit fly (Diptera: tephritidae). J Econ Entomol. 89 (1):104-110

Robbins G. 1989. Intraperitoneal safety test in mice: BMP 144(2X). (3X): Study No. S2032. Unpublished study prepared by Cosmopolitan Safety Evaluation, Inc. 18 p. MRID 40951100

Robbins G. 1991a. BMP technical powder: Intraperitoneal safety test in mice: Lab Project Number: S3101. Unpublished study prepared by Cosmopolitan Safety Evaluation, Inc. 16 p. MRID 41826608

Robbins G. 1991b. BMP technical powder: Intraperitoneal safety test in mice: Lab Project Number: S3102. Unpublished study prepared by Cosmopolitan Safety Evaluation, Inc. 17 p. MRID 41826609

Rodenhouse NL; Holmes RT. 1992. Results of experimental and natural food reductions for breeding black-throuted blue warblers. Ecology. 73: 357-372

Rowell R. 2000. Slurry Manufacturing process for Dipel/Foray Products (*Bacillus thuringiensis* subsp. *kurstaki*) at Abbott Laboratories: Lab Project Number: BTKSLMAN-V28R. Unpublished study prepared by Abbott Laboratories. 27 p. MRID No. 45233003

Salamitou S; Ramisse F; Brehelin M; Bourget D; et al. 2000. The plcR regulon is involved in the opportunistic properties of *Bacillus thuringiensis* and Bacillus cereus in mice and insects. Microbiology. 146: 2825-2832

Sample BE; Butler L; Zivkovich C; Whitmore RC; Reardon R. 1996. Effects of *Bacillus thuringiensis* berliner var. *kurstaki* and defoliation by the gypsy moth on native arthropods in West Virginia. Can Entomol. 128: 573-592

Samples JR; Buettner H. 1983a. Corneal ulcer caused by a biologic insecticide (*Bacillus thuringiensis*). Am J Ophthalmol. 95: 258-260

Samples JR; Buettner H. 1983b. Ocular infection caused by a biological insecticide. J Infectious Dis. 148: 614

Sandoz Crop Protection Corporation. 1988. Submission of chemistry, toxicity and residue data on SAN 418-SC-62 in Support of Trident Biological Insecticide registration. Transmittal of 13 studies. MRID 40497400

Saxena D; Ben-Dov E; Manasherob R; et al. 2002. A UV tolerant mutant of *Bacillus thuringiensis* subsp. *kurstaki* producing melanin. Cur Microbiol. 44(1):25-30

Schindler J. 1990a. Single intraperitoneal administration of *Bacillus thuringiensis* strain SA-3 in mice: SRI Project Number LSC-8491: SRI Study No. 8491-M03-89. Unpublished study prepared SRI International. 15 p. MRID 41441505

Schindler J. 1990b. Single intraperitoneal administration of *Bacillus thuringiensis* strain SA-3A in mice: SRI Project Number LSC-8491: SRI Study No. 8491-M04-89. Unpublished study prepared by SRI International. 16 p. MRID 41441506

Schindler J. 1990c. Single intraperitoneal administration of *Bacillus thuringiensis* strain SA-10 in mice: Lab Project Number: 8491-M05-89: LSC-8491. Unpublished study prepared by SRI International. 15 p. MRID 41441609

Schindler J. 1990d. Single intraperitoneal administration of *Bacillus thuringiensis* strain SA-12 in mice: Lab Project Number: 8491-M07-89: LSC-8491. Unpublished study prepared by SRI International. 17 p. MRID 41441610

Schindler J. 1990e. Single intraperitoneal administration of *Bacillus thuringiensis* strain 313 in mice: Lab Project Number: 891-M01-89: LSC-8491. Unpublished study prepared by SRI International. 15 p. MRID 41441611

Schindler J. 1990f. Single intraperitoneal administration of *Bacillus thuringiensis* strain SA-11 in mice: Lab Project Number: 8491-M06-89: LSC-8491. Unpublished study prepared by SRI International. 15 p. MRID 41441612

Sherwood R. 1989a. EPA Subdivision M Tier I acute pulmonary toxicity/pathogenicity testing of Foil Oil flowable and technical biopesticides: Final Report: IIT Project Number L08245, Study No. 1. Unpublished study prepared by IIT Institute, Life Sciences Research. 44 p. MRID 41308603

Sherwood R. 1989b. Acute intraperitoneal toxicity/pathogenicity testing of Foil technical powder, a microbial pesticide: Final Report: IITRI Project Number L08239: Study No. 7. Unpublished study prepared by IIT Research Institute, Life Sciences Research. 19 p. MRID 41308607

Shindler J. 1990. Single intraperitoneal administration of *Bacillus thuringiensis* strain SA-2 in mice: SRI Project Number LSC-8491: SRI Study No. 8491-MO2-89. Unpublished study prepared by SRI International. 38 p. MRID 41441504

Siegel JP. 2001. The mammalian safety of *Bacillus thuringiensis*-based insecticides. J Invertebr Pathol. 77(1):13-21

Siegel JP; Shadduck JA. 1990. Clearance of Bacillus sphaericus and *Bacillus thuringiensis* ssp. israelensis from mammals. J Econ Entomol. 83:347-355

Siegel JP; Shadduck JA; Szabo J. 1987. Safety of the entomopathogen *Bacillus thuringiensis* var. israelensis for mammals. J Econ Entomol. 80:717-723

Siegel JP; Smith AR; Maddox JV; et al. 1993. Use of cellular fatty acid analysis to characterize commercial brands of *Bacillus thuringiensis* var. israelensis. J Am Mosquito Contr Assoc. 9(3):330-334

Siegel JP; Smith AR; Novak RJ. 2000. Cellular fatty acid analysis of isolates of *Bacillus thuringiensis* serovar *kurstaki*, strain HD-1. Biol Contr. 17:82-91

Simpson W Jr; Schuman SH. 2002. Recognition and management of acute pesticide poisoning. Am Fam Physic. 65(8):1599-1604

Sims SR. 1997. Host activity spectrum of the cryiia *Bacillus thuringiensis* subsp. *kurstaki* protein: effects on lepidoptera, diptera, and non-target arthropods. Southwest Entomol. 22:395-404

Smirnoff WA; MacLeod CA. 1961. Study of the survival of *Bacillus thuringiensis* Berliner in the digestive tracts and in feces of a small mammal and birds. J Invert Pathol. 3:266-270

Smith R; Cooper R. 1990. Vectobac technical powder: Product chemistry based on *Bacillus thuringiensis*, subspecies Israelensis strain AM65-52: (ATCC-SD-1276) as the active ingredient: Lab Project Nos. Abbott Lab-VTp-02: 910-8906. Unpublished study prepared by Abbott Laboratories. 174 p. MRID 41439001

Smith R; Regan K. 1989a. Antibiotic sensitivity patterns for *Bacillus thuringiensis* subsp. Israelensis strain SA3: Final Report No. 89/12/12D. Unpublished study prepared by Sandoz Crop Protection Corp. 38 p. MRID 41441514

Smith R; Regan K. 1989b. Antibiotic sensitivity patterns for *Bacillus thuringiensis* subsp. Israelensis strain SA3A: Final Report No. 89/12/12E. Unpublished study prepared by Sandoz Crop Protection Corp. 38 p. MRID 41441515

Smith R; Regan K. 1989c. Antibiotic sensitivity patterns for *Bacillus thuringiensis* subsp. tenebrionis strain SA10: Lab Project Number: 89/12/12B. Unpublished study prepared by Sandoz Crop Protection Corp. 28 p. MRID 41441621

Smith R; Regan K. 1989d. Antibiotic sensitivity patterns for *Bacillus thuringiensis* subsp. *kurstaki* strain SA11001C98-1-1:Lab Project Number: 89/12/12. Unpublished study prepared by Sandoz Crop Protection Corp. 28 p. MRID 41441622

Smith R; Regan K. 1989e. Antibiotic sensitivity patterns for *Bacillus thuringiensis* subsp. *kurstaki* strain INT-15-313: Lab Project Number: 89/12/12A. Unpublished study prepared by Sandoz Crop Protection Corp. 28 p. MRID 41441623

Smith R; Regan K. 1989f. Antibiotic sensitivity patterns for *Bacillus thuringiensis* subsp. *kurstaki* strain SA12: Lab Project Number: 89/12/12F. Unpublished study prepared by Sandoz Crop Protection Corp. 28 p. MRID 41441624

Smith R; Regan K. 1990g. Biochemical and morphological characteristics of *Bacillus thuringiensis* subsp. Aizawai strain SA2 with a discussion of strain history included: Final Report No. 90/02/02B. Unpublished study prepared by Sandoz Crop Protection Corp. 63 p. MRID 41441501

Smith R; Regan K. 1990h. Biochemical and morphological characteristics of *Bacillus thuringiensis* subsp. Israelensis strain SA3 with a discussion of strain history included: Final Report No. 90/02/02D. Unpublished study prepared by Sandoz Crop Protection Corp. 38 p. MRID 41441502

Smith R; Regan K. 1990i. Biochemical and morphological characteristics of *Bacillus thuringiensis* subsp. Israelensis strain SA3A with a Discussion of strain history included: Final Report No. 90/02/02A. Unpublished study prepared by Sandoz Crop Protection Corp. 63 p. MRID 41441503

Smith R; Regan K. 1990j. Antibiotic sensitivity patterns for *Bacillus thuringiensis* subsp. Aizawai, strain SA2: Final Report No. 89/12/12C. Unpublished study prepared by Sandoz Crop Protection Corp. 28 p. MRID 41441513

Smith R; Regan K. 1990k. Biochemical and morphological characteristics of *Bacillus thuringiensis* subsp. *kurstaki* strain SA12 with a discussion of strain history included: Lab Project No. 90/02/02F. Unpublished study prepared by Sandoz Crop Protection Corp. 63 p. MRID 41441601

Smith R; Regan K. 1990l. Biochemical and morphological characteristics of *Bacillus thuringiensis* subsp. tenebrionis strain SA 10 with a discussion of strain history included: Final Report: Lab Project N0. 90/02/02. Unpublished study prepared by Sandoz Crop Protection Corp. 63p. MRID 41441602

Smith R ; Regan K. 1990m. Biochemical and morphological characteristics of *Bacillus thuringiensis* subsp. *kurstaki* strain INT-15-313 with a Discussion of strain history included:

Final Report: Project No. 90/02/02C. Unpublished study prepared by Sandoz Crop Protection Corp. 63 p. MRID 41441603

Smith R; Regan K. 1990n. Biochemical and morphological characteristics of *Bacillus thuringiensis* subsp.*kurstaki* strain SA11001C98-1-1 with a discussion of strain history included: Final Report: Project No. 90/02/02E. Unpublished study prepared by Sandoz Crop Protection Corp. 63 p. MRID 41441604

Snarski VM. 1990. Interactions between *Bacillus thuringiensis* subsp. *israelensis* and fathead minnows, *Pimephales promelas* Rafinesque, under laboratory conditions. Appl Environ Microbiol. 56:2618-2622

Sopuck L; Ovaska K; Whittington B. 2002. Responses of songbirds to aerial spraying of the microbial insecticide *Bacillus thuringiensis* var. *kurstaki* (Foray 48b) on Vancouver Island, British Columbia, Canada. Environ Toxicol Chem. 21(8):1664-1672

Sorensen E; Rugh S; Overholt J. 1990a. Product analysis data: Analysis of samples and analytical methods in support of reregistration of Biobit Flowable Concentrate: Lab Project No. NOVO/REBF/VOL3. Unpublished study prepared by Novo Nordisk BioIndusrials, Inc. 35 p. MRID 41657002

Sorenson E; Rugh S; Overholt J. 1990b. Product analysis: Biobit Wettable Powder: Lab Project No. NOVO/REBW/VOL3. Unpublished study prepared by Novo Nordisk in cooperation with Novo Nordisk Bioindustrials, Inc. 37 p. MRID 41653901

Stephens L; McClane W; Wooldridge AW; et al. 1975. Effectiveness Data. (Unpublished study received Mar 8, 1976 under 239-EX-79; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:227742-E). MRID 00014931

Stoll R. 1984a. Acute oral LD50 toxicity/infectivity study of Teknar in the rat: Project No. T-1866. Unpublished study prepared by Sandoz, Inc. 25 p. MRID 00142733

Stoll R. 1984b. Acute dermal LD50 toxicity/infectivity study in the rat on Teknar: Project No. T-1867. Unpublished study prepared by Sandoz, Inc. 18 p. MRID 00142734

Sundaram A. 1995. Physical properties and evaporation characteristics of nonaqueous insecticide formulations, spray diluents and adjuvant/co-solvent mixtures. J Environ Sci Hlth Part B Pest Food Contam Agric Wastes. 30(1):113-138

Sundaram K MS; Sundaram A. 1992. An insect bioassay method to determine persistence of *Bacillus thuringiensis* var. *kurstaki* (Btk) protein in oak foliage, following application of a commercial formulation under field and laboratory conditions. J Environ Sci Hlth Part B Pest Food Contam Agric Wastes. 27:73-112

Sundaram A; Sundaram KMS. 1996. Effect of sunlight radiation, rainfall and droplet spectra of sprays on persistence of *Bacillus thuringiensis* deposits after application of dipel 76af formulation onto conifers. J Environ Sci Hlth Part B Pest Food Contam Agric Wastes. 31(5):1119-1154

Sundaram A; Sundaram KMS; Sloane L. 1996. Spray deposition and persistence of a *Bacillus thuringiensis* formulation (Foray 76B) on spruce foliage, following aerial application over a

northern ontario forest. J Environ Sci Health, Part B, Pestic Food Contam Agric Wastes. 31(4):763-813

Sundaram A; Sundaram KMS; Nott R; Curry J; Sloane L. 1997. Persistence of *Bacillus thuringiensis* deposits in oak foliage, afer aerial application of foray 48b using rotary and pressure atomizers. J Environ Sci Health, Part B, Pestic Food Contam Agric Wastes. 32(1):71-105

Surgeoner GA; Farkas MJ. 1990. Review of *Bacillus thuringiensis* var. *kurstaki* (BTK) for use in forest pest management programs in Ontario - with special emphasis on the aquatic environment. Report to the Water Resources Branch, Ontario Ministry of the Environment, Toronto, Canada. 87p. (Cited in USDA 1995).

Surprenant D. 1987a. Static acute toxicity of SAN 418 SC62 (B. t. tenebrionis) to rainbow trout (*Salmo gairdneri*): Report No. 87-10-2520. Unpublished study performed by Springborn Life Sciences, Inc. 18 p. MRID 40497411

Surprenant D. 1987b. Static acute toxicity of SAN 418 SC62 (B. t. tenebrionis) to daphnids (*Daphnia magna*): Report No.87-10-2519. Unpublished study performed by Springborn Life Sciences, Inc. 17 p. MRID 40497412

Swiecicka I; Fiedoruk K; Bednarz G. 2002. The occurrence and properties of *Bacillus thuringiensis* isolated from free-living animals. Lett Appl Microbiol. 34(3):194-198

Swysen C; Hoogkamer P. 1991. The determination of active ingredient content of products based on *Bacillus thuringiensis* var. *kurstaki* and var. *Israelensis* using SDS page electrophoresis: Lab Project No. FN. 5791. Unpublished study prepared by Duphar B.V. and Solvay & Cie. 80 p. MRID 41939901

Tamez-Guerra P; Mcguire MR; Medrano-Roldan H; et al. 1996. Sprayable granule formulations for *Bacillus thuringiensis*. J Econ Entomol. 89:1424-1430

Tapp H; Stotzky G. 1995a. Dot blot enzyme-linked immunosorbent assay for monitoring the fate of insecticidal toxins from *Bacillus thuringiensis* in soil. Appl Environ Microbiol. 61:602-609

Tapp H; Stotzky G. 1995b. Insecticidal activity of the toxins from *Bacillus thuringiensis* subsp. *kurstaki* and subsp.tenebrionis adsorbed and bound on pure and soil clays. Abstr Gen Mtg Am Soc Microbiol. 95(0):406

Tapp H; Stotzky G. 1997. Monitoring the insecticidal toxins from *Bacillus thuringiensis* in soil with flow cytometry. Can J Microbiol. 43:1074-1078

Tapp H; Stotzky G. 1998. Persistence of the insecticidal toxin from *Bacillus thuringiensis* subsp. *kurstaki* in soil. Soil Biol Biochem. 30:471-476

Tayabali AF; Seligy VL. 2000. Human cell exposure assays of *Bacillus thuringiensis* commercial insecticides: Production of bacillus cereus-like cytolytic effects from outgrowth of spores. Environ Health Perspect. 108(10):919-930

Teschke K; Chow Y; Bartlett K; Ross A; Van Netten C. 2001. Spatial and temporal distribution of airborne *Bacillus thuringiensis* var. *kurstaki* during an aerial spray program for gypsy moth eradication. Environ Hlth Perspect. 109(1):47-54.

Theoduloz C; Roman P; Bravo J; et al. 1997. Relative toxicity of native chilean *Bacillus thuringiensis* strains against scrobipalpuloides absoluta (Lepidoptera: gelechiidae). J Appl Microbiol. 82(4):462-468

Thomas WE; Eliar DJ. 1983. *Bacillus thuringiensis* var. israelensis crystal delta-endotoxin: Effects on insect and mammalian cells in vitro and in vivo. J Cell Sci. 60:181-187

Thomas EM; Watson TF. 1990. Effect of Dipel (*Bacillus thuringiensis*) on the survival of immature and adult Hyposoter exiguae (Hymenoptera: Ichneumonidae). J Invertebr Pathol. 47:178-183

Thomas DJI; Morgan JAW; Whipps JM; Saunders JR. 2000. Plasmid transfer between the *Bacillus thuringiensis* subspecies *kurstaki* and tenebrionis in laboratory culture and soil and in lepidopteran and coleopteran larvae. Appl Environ Microbiol. 66: 118-124

Thompson M. 1991a. CGA-237218: A dietary and toxicity study with ladybird beetles: Lab Project No. 108-313. Unpublished study prepared by Wildlife International Ltd. 18 p. MRID 41994320

Thompson M. 1991b. CGA-237218: A dietary and toxicity study with the green lacewing larvae: Lab Project No. 108-312. Unpublished study prepared by Wildlife International Ltd. 18 p. MRID 41994321

Thompson M; Hoxter K; Smith G et al. 1990. BTK Toxbatch NB75 Batch No. PPQ 2843: A dietary pathogenicity and toxicity study with the parasitic Hymnenopteran Pediobus foveolatus in support of registration of Biobit Flowable Concentrate: Lab Project No. 254/115A. Unpublished study prepared by Wildlife International Ltd. MRID 41657013

Thorpe KW; Ridgway RL; Webb RE. 1997. Effectiveness of diflubenzuron and *Bacillus thuringiensis* against gypsy moth populations. North J Appl Forest. 14:135-140

Thorpe KW; Podgwaite JD; Slavicek JM; Webb RE. 1998. Gypsy moth (Lepidoptera: lymantriidae) control with ground-based hydraulic applications of gypchek, *in vitro*-produced virus, and *Bacillus thuringiensis*. J Econ Entomol. 91:875-880

Tompkins G; Engler R; Mendelsohn M; Hutton P. 1990. Historical aspects of the quantification of the active ingredient percentage for *Bacillus thuringiensis* products. *In*: L.A. Hickle & W.L. Fitch (Eds) ACS Symposium Series No. 432 Analytical Chemistry of *Bacillus thuringiensis*. p. 9-13

Tsai SF; Liao JW; Wang SC. 1995. Clearance and distribution of *Bacillus thuringiensis* sub. *kurstaki* from by oral administration. Plant Protect Bull (Taichung). 37(3):265-270

U.S. EPA/OPP (U.S. Environmental Protection Agency/Office of Pesticide Programs). 1998. Reregistration Eligibility Decision (RED): *Bacillus thuringiensis*. Available at: http://cfpub.epa.gov/oppref/rereg/status.cfm?show=rereg

U.S. EPA/OPP. 2000. Pesticide fact sheet: '*Bacillus thuringiensis*' subsp. '*kurstaki*' cryia(c) delta-endotoxin and its controlling sequences as expressed in cotton (Revised). Govt Reports Announcements & Index (GRA&I), Issue 16

U.S. EPA/ORD (U.S. Environmental Protection Agency/Office of Research and Development). 1993. Wildlife Exposure Factors Handbook. Volumes 1 and 2. EPA/600/R-93/187a,b. Pagination not continuous. Available NTIS: PB94-174778 and PB94-174779

U.S. EPA/ORD (U.S. Environmental Protection Agency/Office of Research and Development). 2000. Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. Office of Research and Development, U.S. EPA, Washington, DC. EPA/630/R-00/002. Report dated August2000. Available at www.epa.gov/ncea

USDA (U.S. Department of Agriculture). 1995. Gypsy Moth Management in the United States: A Cooperative Approach. Final Environmental Impact Statement. Appendix F (Human Health Risk Assessment) and Appendix G (Ecological Risk Assessment)

USDA/FS (U.S. Department of Agriculture/Forest Service). 2002. B.t. Usage by State - 1999. www.fs.fed.us/na/morgantown/fhp/gmoth/gm_news47/chart1.htm

Valadares de Amorim G; Whittome B; Shore B; Levin DB. 2001. Identification of *Bacillus thuringiensis* subsp. *kurstaki* strain HD1-Like bacteria from environmental and human samples after aerial spraying of Victoria, British Columbia, Canada, with Foray 48B. Appl Environ Microbiol. 67:1035-1043

Valaitis AP; Jenkins JL; Lee MK; Dean DH; Garner KJ. 2001. Isolation and partial characterization of gypsy moth btr-270, an anionic brush border membrane glycoconjugate that binds *Bacillus thuringiensis* cry1a toxins with high affinity. Arch Insect Biochem Physiol. 46(4):186-200

Valent BioSciences Corporation. 2000a. Submission of Product Chemistry Data in Support of the Application for Registration of *Bacillus thuringiensis* var. aizwaii, Bacillus thurinigiensis var. israelensis, *Bacillus thuringiensis* var. *kurstaki*, *Bacillus thuringiensis* var.tenebrionis. MRID 45136500

Valent Biosciences. 2000b. Submission of Product Chemistry Data in Support of the Application for the Registration of *Bacillus thuringiensis* subsp. aizawai Slurry, Bacillus subsp. israelensis Slurry, Bacillus subsp. *kurstaki* Slurry, Bacillus subsp. tenebrionis Slurry and Baci. MRID 45233000

Van Netten C; Teschke K; Leung V; Chow Y; Bartlett K. 2000. The measurement of volatile constituents in foray 48b, an insecticide prepared from *Bacillus thuringiensis* var. *kurstaki*. Sci Total Environ. 263(1-3):155-160

Vazquez-Padron RI; Gonzales-Cabrera J; Tovar CG; et al. 2000. Cry1ac protoxin from *Bacillus thuringiensis* sp. *kurstaki* HD73 binds to surface proteins in the mouse small intestines. Biochem Biophys Res Commun. 271:54-58

Venette RC; Luhman JC; Hutchison WD. 2000. Survivorship of field-collected european corn borer (Lepidoptera: crambidae) larvae and its impact on estimates of resistance to *Bacillus thuringiensis* berliner. J Entomol Sci. 35:208-212

Visser S; Addison JA; Holmes SB. 1994. Effects of Dipel 176, a *Bacillus thuringiensis* subsp. *kurstaki* (B.t.k.) formulation, on the soil microflora and the fate of B.t.k. in an acid forest soil: a laboratory study. Can J Forest Res. 24:462-471

Vlachos D. 1991. Acute intraperitoneal toxicity/pathogenicity screening studies of technical CGA-237218 in mice: Lab Project Nos. 7961-91: 7963-91: 7965-91. Unpublished study prepared by Stillmeadow, Inc. 103 p. MRID 41994303

WHO (World Health Organization). 1980. Data Sheet on the Biological Control Agent *Bacillus thuringiensis* Serotype H-14 (de Barjac 1978): WHO/VBC/79.750. (Unpublished study; CDL:246969-B). MRID 00096533

WHO (World Health Organization). 1999. *Bacillus thuringiensis*. Environmental health criteria No. 217

Wang C; Strazanac J; Butler L. 2000. Abundance, diversity, and activity of ants (Hymenoptera: formicidae) in oak-dominated mixed Appalachian forests treated with microbial pesticides. Environ Entomol. 29:579-586

Ward T; Boeri R. 1990. Chronic toxicity of vectobac technical material (*Bacillus thuringiensis* var. Israelensis) to the daphnid, *Daphnia magna*: Lab Study No. 9022-A; Method No. IPM-2. Unpublished study prepared by EnviroSystems Div., Resource Analysts, Inc. 46 p. MRID 41439009

Wasano N; Kim KH; Ohba M. 1998. Delta-endotoxin proteins associated with spherical parasporal inclusions of the four lepidoptera-specific *Bacillus thuringiensis* strains. J Appl Microbiol. 84:501-508

Webb RE; Peiffer R; Fuester RW; et al. 1998. An evaluation of the residual activity of traditional, safe, and biological insecticides against the gypsy moth. J Arboricult. 25:286-292

Wencheng Z; Gaixin R. 1998. Study of the bceT and hblA genes and the hemolysin BL of *Bacillus thuringiensis* group. Clin J Microbiol Immunol. 18:428-433

Whaley WH; Anhold J; Schaalje GB. 1998. Canyon drift and dispersion of *Bacillus thuringiensis* and its effect on select nontarget lepidopterans in Utah. Environ Entomol. 27:539-548

Williams WL; Esposito RG; Hernandez HG. 1959a. To determine the effect of intra-peritoneal injection of Lavatrol on Weight gain and mortality of mice. (Unpublished study received Jun 30, 1959 under PP0310; submitted by Nutrilite Products, Inc., Buena Park, Calif.; CDL:090329-B). MRID 00090207

Williams WL; Esposito RG; Hernandez HG. 1959b. To Determine the effect of intraperitoneal injection of Larvatrol-*Bacillus thuringiensis*~Berliner–followed by serial passage of blood intraperitoneally through four consecutive passages in mice. (Unpublished study received Jun 30, 1959 under PP0310; submitted by Nutrilite Products, Inc., Buena Park, Calif.; CDL:090329-C). MRID 00090208

Williams WL; Esposito RG; Hernandez HG. 1977b. To determine the effect of intra-peritoneal injection of Biotrol 10W on weight gain and mortality of mice: Experiment Nutrilite

Products, Inc. #1 (504-1). (Unpublished study received Jan 4, 1977 under 6296-13; submitted by Nutrilite Products, Inc., Buena Park, Calif.; CDL:230811-A). MRID 00066178

Williams WL; Esposito RG; Hernandez HG. 1977b. To determine the effect of intra-peritoneal injection of Biotrol 10W- *Bacillus thuringiensis* Berliner - followed by serial passage of blood intra-peritoneally through four consecutive passages in mice: Experiment Nutrilite Products, Inc. #2 (504-5). (Unpublished study received Jan 4, 1977 under 6296-13; submitted by Nutrilite Products, Inc., Buena Park, Calif.; CDL:230811-C). MRID 00066179

Winter P. 1991. CGA-237218: A dietary pathogenicity and toxicity study with the parasitic Hymenopteran Uga menoni: Lab Project No. 108-311A. Unpublished study prepared by Wildlife International Ltd. 21 p. MRID 41994319

Winter P; Hoxter K; Smith G. 1990. *Bacillus thuringiensis* Var. Israelensis: Strain NB31, Tox Batch PPQ 3044: A dietary pathogenicity and toxicity study with ladybird beetles: Lab Project No. 254-122. Unpublished study prepared by Widlife International Ltd. 19 p. MRID 41842710

Winter P; Hoxter K; Smith G. 1991a. A dietary pathogenicity and toxicity study with the green lacewing larvae: Lab Project No. 297-101A. Unpublished study prepared by Wildlife Biological Products, Inc. 14 p. MRID 41751111

Winter P; Hoxter K; Smith G. 1991b. *Bacillus thuringiensis* var. Israelensis: Strain NB31, Tox Batch PPQ 3044: A dietary pathogenicity and toxicity study with green lacewing larvae: Lab Project No. 254-123. Unpublished study prepared by Widlife International Ltd. 22 p. MRID 41842708

Winter P; Hoxter K; Smith G. 1991c. *Bacillus thuringiensis* var. Israelensis: Strain NB31, Tox Batch PPQ 3044: A Dietary pathogenicity and toxicity study with the parasitic Hymenopteran Uga menoni: Lab Project No. 254-121. Unpublished study prepared by Widlife International Ltd. 22 p. MRID 41842709

Wiwat C; Thaithanun S; Pantuwatana S; Bhumiratana A. 2000. Toxicity of chitinase-producing *Bacillus thuringiensis* ssp. *kurstaki* HD-1 (G) toward plutella xylostella. J Invertebr Pathol. 76(4):270-277

Wraight CL; Zangerl AR; Carroll MJ; Berenbaum MR. 2000. Absence of toxicity of *Bacillus thuringiensis* pollen to black swallow tails under field conditions. Proc Natl Acad Sci. 97(14):7700-7703

Yang CY; Pang JC; Kao SS; Tsen HY. 2003. Enterotoxigenicity and cytotoxicity of *Bacillus thuringiensis* strains and development of a process for cry1ac production. J Agric Food Chem. 51(1):100-105

Young B. 1990. 21-Day Prolonged static renewal toxicity of dipel technical to *Daphnia magna*: Lab Project No. 38417. Unpublished study prepared by Analytical Bio-chemistry Laboratories, Inc. 123 p. MRID 41443407

Youston AA. 1973. Effects of *Bacillus thuringiensis* delta-endotoxin on an insect predator which has consumed intoxicated cabbage looper larvae. J Invert Pathol. 21:312-314

Yu L; Berry RE; Croft BA. 1997. Effects of *Bacillus thuringiensis* toxins in transgenic cotton and potato on *Folsomia candida* (Collembola: Isotomidae) and *Oppia nitens* (Acari: oribatidae). J Econ Entomol. 90(1):113-118

Zhioua E; Heyer K; Browning M; Ginsberg HS; Lebrun RA. 1999. Pathogenicity of *Bacillus thuringiensis* variety *kurstaki* to ixodes scapularis (Acari: ixodidae). J Med Entomol. 36:900-902
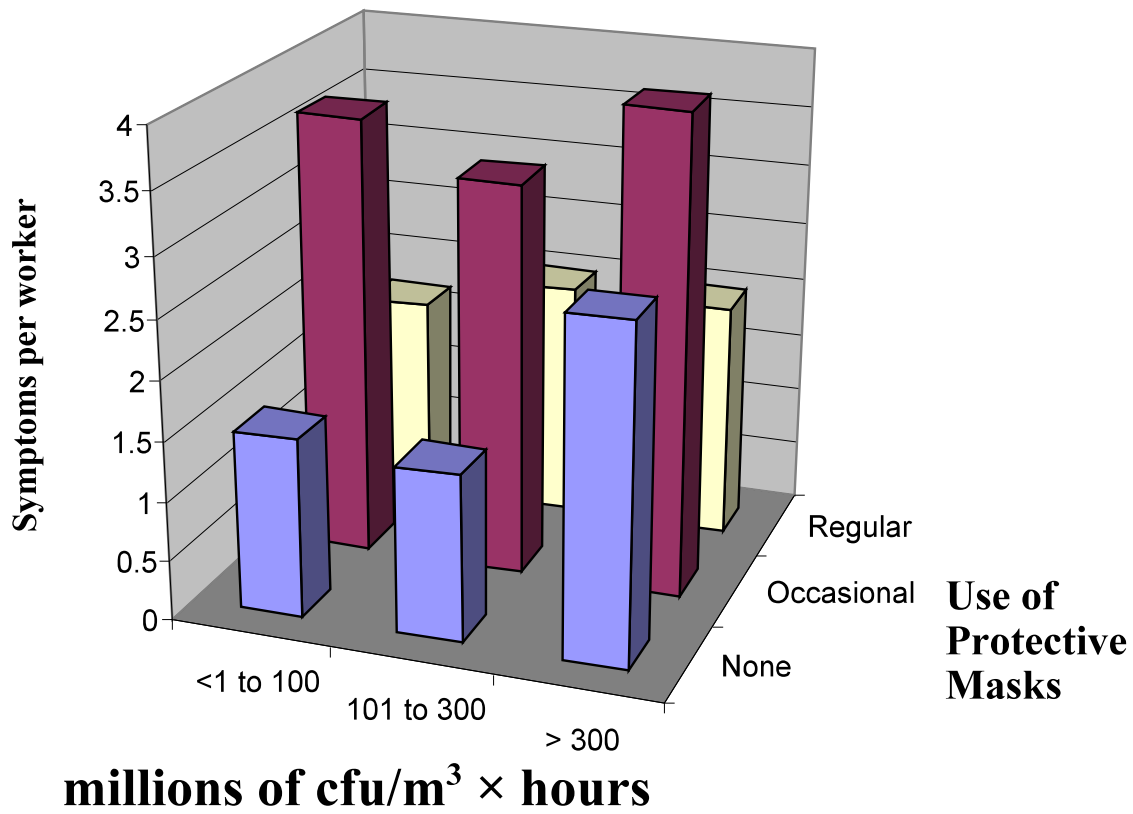
Figure 3-1: Number of symptoms per worker based on total exposure to *B.t.k.* (millions of cfu hours) and the use of protective masks (data from Cook 1994 as summarized in Table 3-6 of this risk assessment)
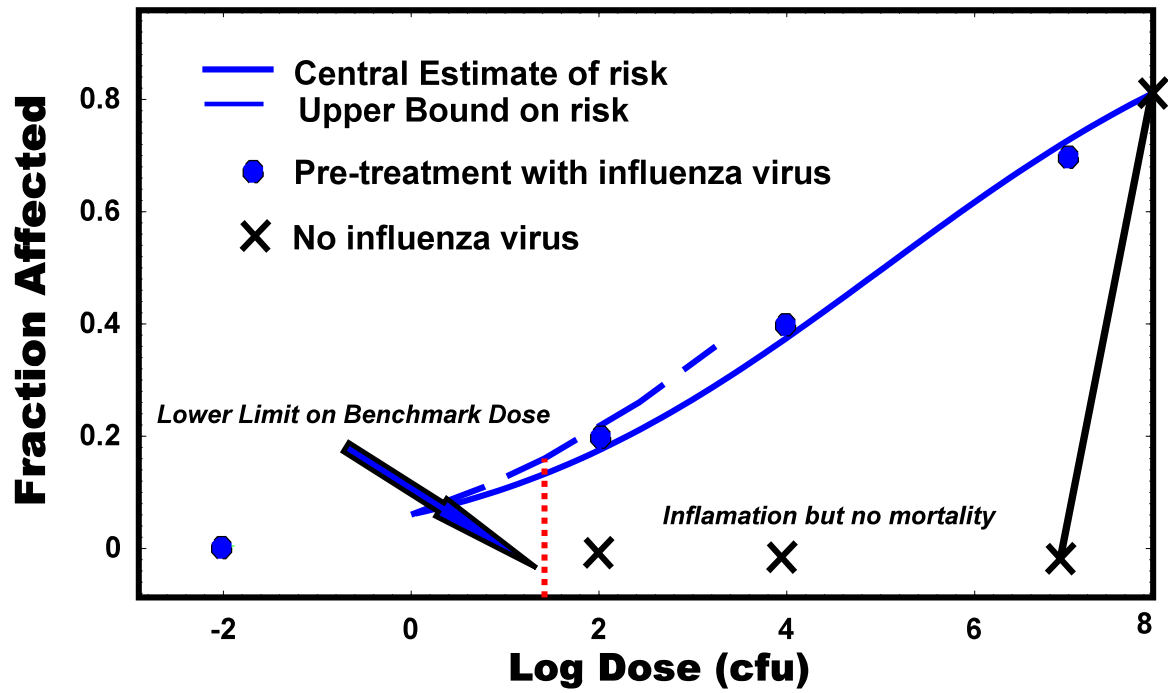
Figure 3-2: Dose-response relationships in mice after intranasal administration of *B.t.k.* with or without previous challenge with influenza virus at 4% of the LD_{50} (data from Hernandez et al. 1999 and 2000).
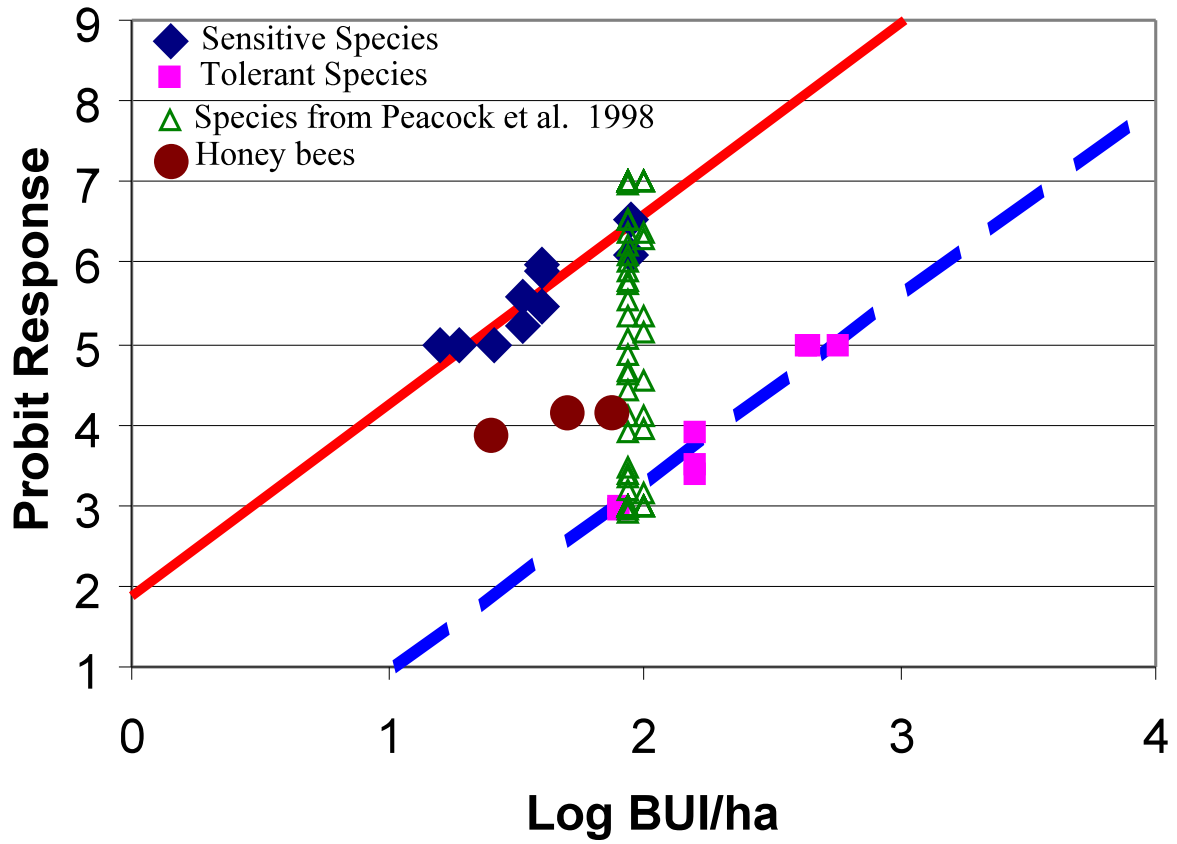
Figure 4-1: Dose-Response Assessment for non-target terrestrial invertebrates.
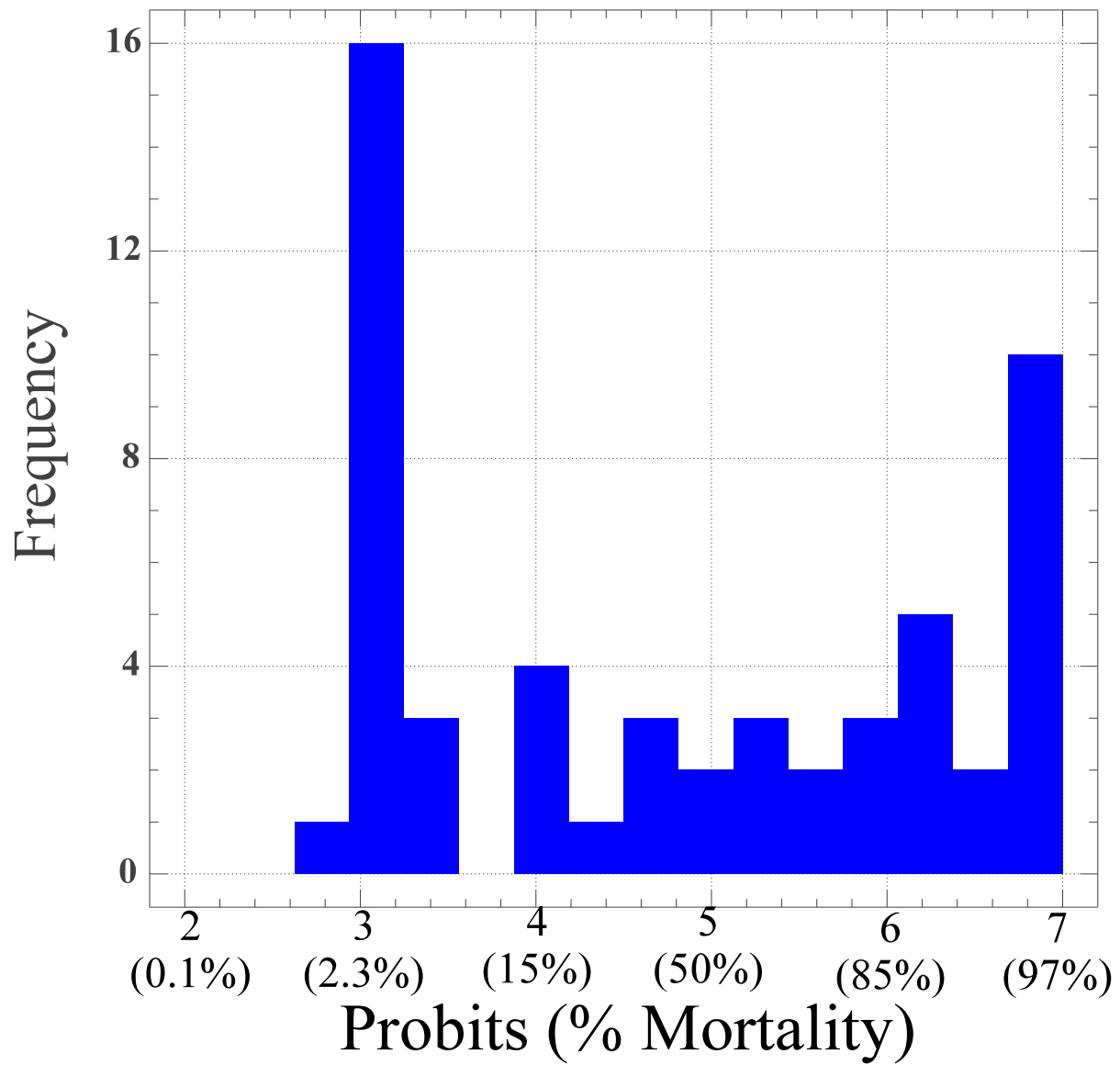
Figure 4-2: Distribution of sensitivity in various non-target lepidoptera (data from Peacock et al. 1998)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[1] Based on a breathing rate of 0.0014 L/hour for a 0.020 g mouse, derived from U.S. EPA (1988a), Recommendations for and Documentation of Values for Use in Risk Assessment, Table 1-3, p. 1-11: L/day = 1.99 $_{Bwkg}$$^{1.0496}$. Note that 0.0014 L/hour is equivalent to 0.0000014 m^3/hour [1 m^3 = 1000 L] or 0.0000336 m^3/day.

[2] cfu/mouse × 70 kg/0.02 kg.

[3] Based on a human breathing rate for moderate activity of 2.5 m^3/hour from U.S. EPA (1989d), Exposure Factors Handbook, Table 3-1, p. 3-4.

[4] From Hernandez et al. (1999, 2000), intranasal instillations in mice without exposure to influenza virus.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

APPENDICES

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

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

Appendix 1-1

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

Appendix 1-2

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

Appendix 1-3

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

Appendix 1-4

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

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

Appendix 2-1

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

Appendix 2-2

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

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

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

Appendix 3: Toxicity in Non-target Lepidoptera			
Product	Species/Exposure	Observations	Reference
B.t.k. (Foray 48B)	Karner blue butterlfy (*Lycaeides melissa samuelis*) larvae (early and late instars) reared on wild lupine foliage treated in laboratory bioassay with *B.t.k.* at rate of 30-37 or 90 BIU/ha for 7 days. A concurrent laboratory bioassay involving gypsy moth 2nd instars on similarly treated white oak for 7 days.	Survival rates for Karner blue larvae were: 100% for controls, 27% at 30-37 BIU/ha treatment rate, and 14% at 90 BIU treatment rate. Survival rates for gypsy moth larvae were: 80% for controls; 33% for low-dose treatment, and 5% for high-dose treatment. Investigators conclude that the Karner blue is both phenologically and physiologically susceptible to *B.t.* used for gypsy moth suppression, although the larval generation at risk and extent of phenological overlap may vary from year to year.	Herms et al. 1997
B.t.k. (Dipel: wettable powder)	Mulberry silkworm (*Bombyx mori*) larvae exposed to laboratory concentrations of 1×10, 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8, or 1×10^9 spore/mL applied to mulberry leaves	$LC_{50} = 1.40\times10$ spores/L (larval instar I) $LC_{50} = 4.20\times10^2$ spores/L (larval instar II) $LC_{50} = 1.0\times10^3$ spores/L (larval instar III) $LC_{50} = 2.0\times10^5$ spores/L (larval instar IV) $LC_{50} = 6.3\times10^6$ spores/L (larval instar II) Larval mortality was dose-dependent with highest % mortality observed at highest concentrations of *B.t.* The highest % of mortality was observed in the early instars, compared with the later instars, and a longer incubation period was observed at the lower concentrations. The higher concentrations of *B.t.* were associated with decreased pupation, greater pupal mortality, increased incidences of malformed adult emergence and lower emergence of normal adults in all instars.	Jayanthi and Padmavathamma 1997

Appendix 3: Toxicity in Non-target Lepidoptera			
Product	Species/Exposure	Observations	Reference
B.t.k. (Foray 48B)	Swallowtail butterflies (*Papilio glaucus* and *Papilio canadensis*) and promethea moth (*Callosamia promethea*) (1st and 2nd instars of the three nontarget species) exposed to Foray 48B applied at a rate of 40 BIU/ha to individual trees using a *B.t.-dedicated* backpack sprayer to eliminate possibility of contamination from other insecticides. Larvae were placed on the tree at 0 or 1 day after spray and monitored for 7-8 days.	Significant differences in larval survival by day 5 between sprayed and control trees; nearly all larvae died or disappeared by day 8 from sprayed foliage. See text for additional details.	Johnson et al. 1995
B.t.k. (Foray 48B)	Long-term persistence field studies in which Foray 48B was applied at a rate of 40 BIU/ha to 5-year-old, 1-2 m high potted tulip trees which were randomly assigned to full sun or below-canopy locations in the field sites.	Tree survival was lower in the below-canopy locations, but the differences were not always significant. Toxicity toward early instar *P. glaucus* persisted for up to 30 days.	Johnson et al. 1995
Dipel 8AF	Laboratory bioassays equivalent to application rate of 89 BIU/ha.	18 species of lepidoptera native to U.S. 8 species of larvae (44%) evidenced significant mortality.	Peacock et al. 1998

See text and Tables 4-1 and 4-2 to additional details. |
Foray 48B	Laboratory bioassays equivalent to application rate of 99 BIU/ha.	42 species of lepidoptera native to U.S. 27 species of larvae (61%) evidenced significant mortality.	
Foray 48F	Field study in which Foray 48F was applied at a rate of 40 BIU/acre in May of 1997 and 1998 to two forests susceptible to gypsy moth. Nontarget lepidoptera monitored in two pre-treatment year as well as in treatment years.	Larvae of three lepidopteran species were significantly decreased in treatment years: *Lambdina fervidaria* [geometrid], *Heterocampa guttivitta* [notodontid], and *Achatia distincta* [noctuid]. For 19 other species, larval counts were significantly higher in treatment years as were the total number of noctuids combined and the total number of all nontarget lepidopteran species combined.	Rastall et al. 2003
Dipel 6AF (12,000 IU/mg)	Applied aerially at 59 BIU/ha (ca. 24 BIU/acre).	Two non-target lepidoptera: *Incisalia fotis* (Desert Elfin butterfly) and *Callophrys sheridanii* (Sheridan's Hairstreak butterfly). Significant mortality in larvae that was dose-related. 3,473 cfu/mm^2 lead to nearly 80% mortality in 7 days.	Whaley et al. 1998

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

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

Appendix 4-1

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

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

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

Appendix 5-1

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

Appendix 6: Toxicity of *B.t.k.* and *B.t.k.* Formulations to Aquatic Invertebrates (sorted by specified group – phylum, order, or subclass – followed by studies on mixed populations).

Cladocera

Dipel, NOS	*Daphnia magna*, 21-day static renewal, 0, 5, 50, and 100 mg/L. Constant aeration.	Increased BOD of test chambers at 50 and 100 mg/L. 21 Day EC_{50} of 14 mg/L based on immobilization. Delayed in time to first brood and number of young per adult at 5 mg/L.	Young 1990
B.t.k. CGA-237218 [Specified as containing 1.06×10^{11} cfu/g equivalent to 1.06×10^{8} cfu/mg].	*Daphnia magna*, 21-day static renewal. Measured concentrations of 0, 4.85×10^{7}, 1.57×10^{8}, 6.24×10^{8}, 1.77×10^{9}, 5.71×10^{9} cfu/L. Aeration not specified. These concentrations are equivalent to about 0, 0.45, 1.4, 5.9, 17, and 54 mg/L.	No daphnids survived at two highest concentrations. Decreased survival at three lower concentrations: 85% (low), 10% (mid), and 30% (high). Decrease significant only at mid-concentration group. No difference in reproduction at the two lower concentrations. Substantial decreases in dissolved oxygen at two highest concentrations [Table 1, p. 28/90].	Christensen 1991d

Copepoda

B.t.k. technical material	*Amphiascus minutus* (copepod). 5, 50, and 500 mg/kg sediment for 10 days. (1×10^{5}, 1×10^{6}, and 1×10^{7} cfu/g sediment)	No adverse effects at any concentration on survival or reproduction. Number of offspring at 500 mg/kg was significantly greater than controls, probably due to the utilization of *B.t.k.* as a food source.	Chandler 1990b; Abbott Labs 1992

Glass Shrimp (Palaemonetes)

Dipel technical material	Grass shrimp (n=60), 30-day static renewal, 100X EEC in water and food: 2.87×10^{9} cfu/L and 2.87×10^{9} cfu/g food.	One shrimp died in both exposed and control groups. No significant differences in body weight or length. No apparent adverse effects.	Christensen 1990h
B.t.k. CGA-237218	Grass shrimp (n=60), 30-day static renewal, dietary: 1.58×10^{10} cfu/g food. Concentration characterized as 200,000 EEC.	Mortality of 12/60 in treatment groups and 14/60 in control group. No effect on survival or growth. No signs of infectivity or pathogenicity.	Christensen 1991f

Appendix 6-1

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

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

Appendix 6-3

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

Appendix G
Gypchek
(Nucleopolyhedrosis Virus)
Risk Assessment
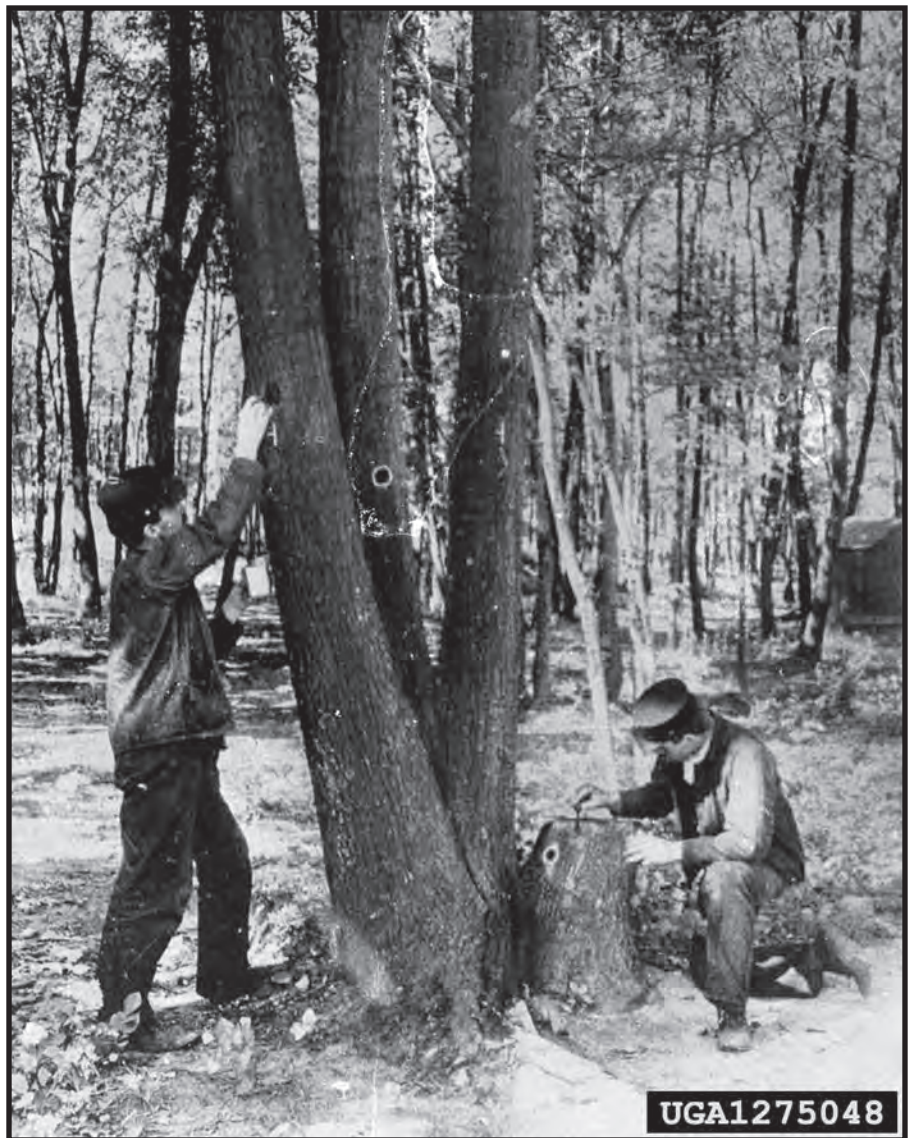
Figure G-1. Creosote was used in 1895 to treat gypsy moth egg masses.

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

Prepared for:

USDA, Forest Service
Forest Health Protection

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Submitted to:
Dave Thomas, COTR
Forest Health Protection Staff
USDA Forest Service
Rosslyn Plaza Building C, Room 7129C
1601 North Kent Street
Arlington, VA 22209

Submitted by:
Patrick R. Durkin
Syracuse Environmental Research Associates, Inc.
5100 Highbridge St., 42C
Fayetteville, New York 13066-0950
Telephone: (315) 637-9560
Fax: (315) 637-0445
E-Mail: SERA_INC@msn.com
Home Page: www.sera-inc.com

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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 (µg/cm^2)	11.21
pounds per gallon (lb/gal)	grams per liter (g/L)	119.8
square centimeters (cm^2)	square inches (in^2)	0.155
square centimeters (cm^2)	square meters (m^2)	0.0001
square meters (m^2)	square centimeters (cm^2)	10,000
yards	meters	0.9144

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

CONVERSION OF SCIENTIFIC NOTATION

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

EXECUTIVE SUMMARY

OVERVIEW

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 $\div 2 \times 10^9$ PIBs/larva]. At the higher application rate, the corresponding value is 500 larvae/acre [$1 \cdot 10^{12}$ PIB/acre $\div 2 \times 10^9$ 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.*
> > <div align="right">–U.S. EPA, 1996, pp. 24-25</div>

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 $\times$ 0.066 lb/acre = 5.61 mg/kg] to 16 mg Gypchek/kg vegetation [240 mg pesticide/kg vegetation per lb/acre $\times$ 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 $\times$ (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 $\div$ 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 $\times$ 14.2 kg vegetation $\div$ 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 $\times$ 14.2 kg vegetation $\div$ 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 $\div$ 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 $\div$ 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 $\times$ 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 $\times$ 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 $\times$ 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 $\times$ 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.*

A.G. Scientific, Inc. 2003. Blankophor, Phorwhite BBU. Information available on-line at: www.agscientific.com/Item/B1003.htm.

Abrahamson LP; Eggen DA; Palm CE. 1979. Gypsy moth suppression tactics and their effects on parasitism and the natural occurrence of nuclear polyhedrosis virus (NPV). AFRI Research Note 29, Applied Forestry Research Institute, State University of NY. (29):1-3.

Adamson A. 1991. Gypchek: Product Identity; Manufacturing Process and Quality Control. Unpublished study prepared by Espro, Inc. 7 p. MRID 41893401.

Agricultural Genetics Co Ltd. 1987. Submission of Toxicological Data to Support the New Application for EUP for Microbial Insecticide, AGC 200. (*Cydia pomonella* granulosis virus of the Codling Moth). Transmittal of 15 studies. MRID 40487300.

Barber KW; WJ Kaupp; SB Holmes. 1993. Specificity testing of the nuclear polyhedrosis virus of the gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae). Canadian Entomologist. 125:1055-1066.

Becker J; Parke GSE. 1976a. Report: The Effects of Insect Virus*L. dispar* NPV Bioserv Lot # 33 on the Eye Mucosa of New Zealand Albino Rabbits. Laboratory No. 6E-2616. (Cannon Laboratories, Inc. for U.S. Forest Service, Insect & Disease Laboratory; unpublished study; CDL:230162-G) MRID 00060696.

Becker J; Parke GSE. 1976b. Dermal Toxicity Study of Insect Virus (*L. dispar* NPV Bioserv Lot 33) in New Zealand Rabbits. Laboratory No. 6E-2617. (Cannon Laboratories, Inc. for U.S. Forest Service; unpublished study; CDL:230162-E) MRID 00060694.

Becker J; Parke GSE. 1976c. Report: The Effects of Insect Virus *L. dispar* NPV Bioserv Lot #33 on the Eye Mucosa of New Zealand Albino rabbits. Laboratory No. 6E-2872. (Cannon Laboratories, Inc. for U.S. Forest Service, Insect & Disease Laboratory; unpublished study; CDL:231360-H) MRID 00068403.

Becker J; Parke GSE; Offenkrantz FM. 1976. Dermal Toxicity Study of Insect Virus (*L. dispar* NPV Bioserv Lot 33) in New Zealand Rabbits. Laboratory No. 6E-2617. (Cannon Laboratories, Inc. for U.S. Forest Service; unpublished study; CDL:227336-Q) MRID 00066101.

Bell MR; Romine CL. 1986. *Heliothis virescens* and *H. zea* (Lepidoptera: Noctuidae): dosage effects of feeding mixtures of Bacillus thuringiensis and a nuclear polyhedrosis virus on mortality and growth. Environmental Entomology. 15(6): 1161-1165.

Brown RM. 1976. Acute Inhalation Toxicity of *L. dispar* NPV Lot # 33 (Insect Virus). Laboratory No. 6E-2619. (Cannon Laboratories, Inc. for U.S. Forest Service; unpublished study; CDL: 230162-F) MRID 00060695.

Brown SE; Kaczmarek FS; DuBois NR; et al. 1978. Comparative properties of the inclusion body proteins of the nucleopolyhedrosis viruses of *Neodiprion sertifer* and *Lymantria dispar*. Archives of Virology 59:319-329.

Brown SE; Kaczmarek FS; Dubois NR; et al. 1979. Comparative properties of the inclusion body proteins of the nucleopolyhedrosis viruses of *Neodiprion sertifer* and *Lymantria dispar*. Archives of Virology 59: 319-329.

Cantwell GF; Knox DA; Lehnert T; et al. 1966. Mortality of the honey bee, *Apis mellifera* in colonies treated with certain biological insecticides. J Invert Pathology. 8(2):228-233.

Cantwell GE; Lehnert T; Fowler J. 19??. Are biological insecticides harmful to the honey bee. American Bee Journal. 112(7): 255-258.

Chou CM; Huang CJ; Lo CF; Kou GH; Wang CH 1996. Characterization of *Perina nuda* Nucleopolyhedrovirus Polyhedrin Gene. J. Invert. Pathol. 67 (3):259-66.

Cook SP; Webb RE; Thorpe KW; Podgwaite JD; White GB. 1997. Field examination of the influence of azadirachtin of gypsy moth (Lepidoptera: lymantriidae) nuclear polyhedrosis virus. Journal of Economic Entomology. 90 (5): 1267-1272.

Cunningham JC; Kaupp WJ; Fleming RA; Brown KW; Burns T. 1993. Development of nuclear polyhedrosis virus for control of gypsy moth (Lepidoptera: Lymantriidae) in Ontario. II. Reduction in dosage and emitted volume (1989 and 1990). Canadian Entomologist. 125: 489-498.

DeBlois RW; Uzgiris EE; Cluxton DH; et al. 1978. Comparative measurements of size and polydispersity of several insect viruses. Analytical Biochemistry 90: 273-288.

DeBlois RW; Uzgiris EE; Cluxton DH; et al. 1978. Comparative measurements of size and polydispersity of several insect viruses. Analytical Biochemistry 90: 273-288.

Doane CC. 1967. Bioassay of nuclear-polyhedrosis virus against larval instars of the gypsy moth. J Invert Pathol. 9:376-386.

Doane CC. 1970. Primary pathogens and their role in the development of an epizootic in the gypsy moth. J Invert Pathol. 15:21-33.

Doane CC. 1976a. Ecology of pathogens of the gypsy moth. In: Prospectives in forest entomology (proceedings). Anderson, J.F.; Kaya, H.K. (eds). Connecticut Agricultural Experiment Station, New Haven, CT (NY Academic Press), pp. 285-296.

Doane CC. 1976b. Epizootiology of diseases of the gypsy moth. Proceedings of the First International Colloquium on Invertebrate Pathology and Ninth Annual Meeting of the Society of Invertebrate Pathology; p. 161-165.

Doane CC; McManus ML. 1981. The gypsy moth: Research toward integrated pest management. USDA Tech Bull 1584. 757 p.

Döller G. 1985. The safety of insect viruses as biological control agents. Biological Insecticides for Biological Control. pp. 399-439.

Drottar KR; Krueger HO. 2001. Carrier 038-A: A 96-hour static acute toxicity test with the rainbor trout (*Oncorhynchus mykiss*). Wildlife International, Ltd. Project Number: 307A-106. U.S. EPA OPPTS Number 850.1075. Report dated December 17, 2001. Copy courtesy of Joseph Cook, USDA/FS.

Durkin PR. 1994. Comparison and Summary of Human Health Risk Assessments for the USDA Control and Eradication Programs. In Proceedings of the 1994 Annual Gypsy Moth Review, D.H. Hilburn, K.J.R. Johnson, and A.D. Mudge (eds), U.S. Department of Agriculture, Salem, Oregon, pp. 170-182.

Durkin PR; Diamond G. 2002. Neurotoxicity, Immunotoxicity, and Endocrine Disruption with Specific Commentary on Glyphosate, Triclopyr, and Hexazinone. Final Report. SERA TR 01-43-08-04a dated January 30, 2002. Available at www.fs.fed.us/foresthealth/pesticide/risk.htm.

Espro Inc. 1991. Submission of Data To Support Gypchek Registration: Product Chemistry Studies. Transmittal of 1 study. MRID 41893400.

Fletcher JS; Nellessen JE; Pfleeger TG. 1994. Literature review and evaluation of the EPA food-chain (Kenega) nomogram, an instrument for estimating pesticide residues on plants. Environ. Toxicol. Chem. 13(9):1383-1391.

Flexner JL; Lighthart B; Croft BA. 1988. The effects of microbial pesticides on non-target beneficial arthropods. Agriculture Ecosystems and Environment. 16:203-254.

Galipeau PR. 1975. A Progress Report on the Effect of Nuclear Polyhedrosis Virus (NPV) on Selected Avian Predators of the Gypsy Moth. (Unpublished study received Jan 11, 1977 under 27586-EX-8; submitted by U.S. Dept. of Agriculture, Forest Service; CDL:230162-S) MRID 00060706.

Gordon EB; Kinsel DA. 1977. Final Report: Primary Eye Irritation and Corrosiveness Study in Rabbits. LBI Project No. 2802 (Litton Bionetics, Inc. for U.S. Forest Service, Northeastern Forest Experiment Station, Forest Insect & Disease Laboratory; unpublished study; CDL:231360-I) MRID 00068404.

Groner A. 1990. Safety to nontarget invertebrates of baculoviruses. In: Laird M; Lacey L A; Davidson EW. eds. Safety of microbial insecticides. Boca Raton: CRC Press, Inc. 135-147 .

Hamlen RA; Yendol WG. 1972. Field Studies on the Persistence of *Porthetria dispar* (L.) Nuclear Polyhedrosis Virus Formulations. (Unpublished study received Jan 11, 1977 under 27586-EX- 8; prepared by Pennsylvania State Univ., Pesticide Research Laboratory and Graduate Study Center and Dept. of Entomology, submitted by U.S. Dept. of Agriculture, Forest Service, Washington, D.C.;.

Hart ER. 1975a. Subacute Toxicity Study--Dogs: Nucleopolyhedrosis Virus (NPV) of the Gypsy Moth. LBI Project Nos. 2241 and 2423; Contract No. 12-14-110-4145-33. (Litton Bionetics, Inc. for U.S. Forest Service, Northeastern Forest Experiment Station, Forest Insect and Disease Laboratory; unpublished study; CDL: 227336-V) MRID 00067103.

Hart ER. 1975b. Final Report: 2-Year Carcinogenicity Study in Rats. LBI Project No. 2241; Contract No. 12-14-110-4145-33. (Litton Bionetics, Inc. for U.S. Dept. of Agriculture, Forest Service, NEFES, Forest Insect and Disease Laboratory, unpublished study; CDL:223573-H) MRID 00049267.

Hart ER. 1976. Final Report: Acute Toxicity of the Nucleopolyhedrosis Virus (NPV) of the Gypsy Moth by Gastric Intubation in Rats. LBI Project No. 2708. (Litton Bionetics, Inc. for U.S. Forest Service, Northeastern Forest Experiment Station, Forest Insect and Disease Laboratory; unpublished study; CDL:231360-E) MRID 00068401.

Hart ER; Cockrell BY. 1975. Final Report: Two Yr. Carcinogenicity Study in Rats. LBI Project No. 2241; Contract No. 12- 14-110-4145-33. (Litton Bionetics, Inc. for U.S. Forest Service, Northeastern Forest Experiment Station, Forest Insect and Disease Laboratory; unpublished study; CDL:230162-K) MRID 00060699.

Hart ER; Thornett HD. 1975a. Eye Irritation-- Rabbits. Final Report: LBI Project No. 2515. (Litton Bionetics, Inc. for U.S. Dept. of Agriculture, Forest Service, NEFES, Forest Insect and Disease Laboratory, unpublished study; CDL:223573-E) MRID 00049264.

Hart ER; Thornett HD. 1975b. Final Report: Primary Skin Irritation--Rabbits. LBI Project No. 2515. (Litton Bionetics, Inc. for U.S. Forest Service, Northeastern Forest Experiment Station, Forest Insect and Disease Laboratory; unpublished study; CDL: 227336-U) MRID 00066104 .

Hart ER; Thornett HD. 1975c. Acute Oral Toxicity--Rats. Final Report: LBI Project No. 2515. (Litton Bionetics, Inc. for U.S. Dept. of Agriculture, Forest Service, NEFES, Forest Insect and Disease Laboratory, unpublished study; CDL:223573-C) MRID 00049262.

Hart ER; Thornett HD. 1975d. Acute Dermal Toxicity--Guinea Pigs. Final Report: LBI Project No. 2515. (Litton Bionetics, Inc. for U.S. Dept. of Agriculture, Forest Service, NEFES, Forest Insect and Disease Laboratory, unpublished study; CDL:223573-D) MRID `.

Hart ER; Thornett HD. 1975e. Primary Skin Irritation--Rabbits. Final Report: LBI Project No. 2515. (Litton Bionetics, Inc. for U.S. Dept. of Agriculture, Forest Service, NEFES, Forest Insect and Disease Laboratory, unpublished study; CDL:223573-F) MRID 00049265.

Hart ER; Thornett HD. 1975g. Final Report: Eye Irritation-- Rabbits. LBI Project No. 2515. (Litton Bionetics, Inc. for U.S. Forest Service, Northeastern Forest Experiment Station, Forest Insect and Disease Laboratory; unpublished study; CDL: 230162-P) MRID 00060704.

Hart ER; Wosu NJ. 1975. Subacute Toxicity Study in Dogs. Contract No. 12-14-110-4145-33. Final rept. (Litton Bionetics, Inc. for U.S. Forest Service, Northeastern Forest Experiment Station, Forest Insect & Disease Laboratory; unpublished study; CDL:230162-J) MRID 00060698.

Hart ER; Thornett HD; Valerio MG. 1975a. Final Report: Acute Oral Toxicity--Rats. LBI Project No. 2515. (Litton Bionetics, Inc. for U.S. Forest Service, Northeastern Forest Experiment Station, Forest Insect and Disease Laboratory; unpublished study; CDL:230162-N) MRID 00060702.

Hart ER; Thornett HD; Valerio MG. 1975b. Final Report: Acute Dermal Toxicity--Guinea Pigs. LBI Project No. 2515. (Litton Bionetics, Inc. for U.S. Forest Service, Northeastern Forest Experiment Station, Forest Insect and Disease Laboratory; unpublished study; CDL:230162-O) MRID 00060703.

Ignoffo CM. 1992. Environmental factors effecting persistence of entomopathogens. Florida Entomologist. 75(4):516-525.

Imlay P; Terrell Y. 1978. The Effects of LDP 53 on the Eye Mucosa of New Zealand Albino Rabbits. Laboratory Nos. 7E-9563 & 7E-9562. (Cannon Laboratories, Inc. for U.S. Forest Service; unpublished study; CDL:232889-A) MRID 00091124.

Kim M-K; Sisson G; Stoltz D. 1996. Ichnovirus infection of an established gypsy moth cell line. J Gen Virology. 77: 2321-2328.

Knox DA. 1970. Tests of certain insect viruses on colonies of honeybees. Journal of Invertebrate Pathology 16(1):152.

Kreutzweiser DP; Ebling PM; Holmes SB. 1997. Infectivity and Effects of Gypsy Moth and Spruce Budworm Nuclear Polyhedrosis Viruses Ingested By Rainbow Trout. Ecotoxicol Environ Saf. 38 (1):63-70.

Kuhn J. 1997a. Primary Eye Irritation Study in Rabbits: Gypchek TGAI. Lab Project Number: 3255-97. Unpublished study prepared by Stillmeadow, Inc. 18 p MRID 44354301.

Kuhn J. 1997b. Primary Eye Irritation Study in Rabbits: Gypchek Solution 2X. Lab Project Number: 3256-97. Unpublished study prepared by Stillmeadow, Inc. 16 p MRID 44354302 .

Lautenschlager RA; Podgwaite J. 1977. Passage of infectious nuclear polyhedrosis virus through the alimentary tracts of two small mammal predators of the gypsy moth, *Lymantria dispar*. Environmental Entomology. 6(5): 737-738.

Lautenschlager RA; Podgwaite JD. 1978. Differential Survival and Passage Rates of Nucleopolyhedrosis Virus through the Alimentary Tracts of Avian and Mammalian Predators of the Gypsy Moth, *Lymantria dispar* L. (U.S. Forest Service, Northeastern Forest Experiment Station; unpublished study; CDL:232891-B) MRID 00090599.

Lautenschlager RA; Podgwaite JD. 1979b. Passage of nucleopolyhedrosis virus by avian and mammalian predators of the gypsy moth *Lymantria dispar*. Environmental Entomology. 8(2):210-214.

Lautenschlager RA; Podgwaite JD. 1979b. Response of birds to aerial application of the nucleopolyhedrosis virus of the gypsy moth, *Lymantria dispar*. Environmental Entomology 8:760-764.

Lautenschlager RA; Rothenbacher H; Podgwaite JD. 1976a. The Response of Small Mammals to an Aerial Application of the Nuclear Polyhedrosis Virus of the Gypsy Moth, *Lymantria dispar* L. (Unpublished study received Jan 11, 1977 under 27586-EX-8; prepared by Northeastern Forest Experiment Station, Forest Insect and Disease Laboratory in cooperation with Pennsylvania State Univ., Dept. of Veterinary Pathology, submitted by U.S. Dept. of.

Lautenschlager RA; Rothenbacher H; Podgwaite JD. 1976b. The Response of Birds to Aerially Applied Nuclear Polyhedrosis Virus of the Gypsy Moth, *Lymantria dispar* L. (U.S. Forest Service, Northeastern Forest Experiment Station, Forest Insect and Disease Laboratory and Pennsylvania State Univ., Dept. of Veterinary Pathology for USFS; unpublished study; CDL:227336-AN) MRID 00066108.

Lautenschlager R; Kircher C; Podgwaite J. 1977. Effect of nucleopolyhedrosis virus on selected mammalian predators of the gypsy moth. (Forest Service research paper NE-377; also In unpublished submission received Sep 11, 1979 under 27586-2; submitted by U.S. Forest Service, Washington, DC; CDL:240994-D) MRID 00134314.

Lautenschlager R; Rothenbacher H; Podgwaite J. 1978a. Response of small mammals to aerial applications of the nucleopolyhedrosis virus of the gypsy moth, *Lymantria dispar*. Environmental Entomology. 7(5): 676-684.

Lautenschlager R; Podgwaite J; Rothenbacher H. 1978b. Effects of field application of gypsy moth *Lymantria dispar* L. (Lepidoptera: Lymantriidae) nucleopolyhedrosis virus (Baculovirus) on birds. Journal of the New York Entomological Society LXXXVI (4)303-304. (Abstract; also In unpublished submission received Sep 11, 1979 under 27586-2; submitted by U.S. Forest Service, Washington, DC; CDL:240994-F) MRID 00134316.

Lautenschlager RA; Podgwaite JD; Watson DE. 1980. Natural occurrence of the nucleopolyhedrosis virus of the gypsy moth *Lymantria dispar* Lepidoptera Lymantriidae in wild birds and mammals. Entomophaga. 25(3):261-267.

Lewis FB. 1971. Mass propagation of insect viruses with specific reference to forest insects. Proceedings, International Collog. Insect Pathology IV:320-326. (Also in unpublished submission received Feb 22, 1980 under 27856-EX-25; submitted by U.S. Dept. of Agriculture, Forest Service, Washington, D.C.; CDL:241872-C) MRID 00030815.

Lewis FB; Mcmanus ML; Schneeberger NF. 1979. Guidelines for the use of Gypchek to control the gypsy moth. NTIS/PB81-216418.

Magnoler A. 1970. Susceptibility of gypsy moth larvae to *Lymantria* spp. nuclear and cytoplasmic-polyhedrosis viruses. Entomophaga 15:407-412.

Maramorosch K; Chase T Jr; Padhi S. 1976. Gypsy Moth Virus: Enzyme and Specificity Studies. Study No. 680-15-1. Prelim. progress rept. (Unpublished study received Jan 11, 1977 under 27586-EX-8; prepared by Rutgers Univ., submitted by U.S. Dept. of Agriculture, Forest Service, Washington, D.C.; CDL:230162-R) MRID 00060705.

Marmorosch K; Chase T Jr; Padhi SB. 1977. Gypsy Moth Virus; Enzyme and Specificity Studies. Study No. 680-15-1. (Unpublished study received Aug 18, 1977 under 27582-2; submitted by Parker Livestock Supply, Inc., Fremont, Nebr.; CDL:231360-L) MRID 00068407 .

Mazzone HM. 1962?. Determination of the Quantity of Nucleopolyhedra in Gypsy Moth Viral Preparations. (U.S. Dept. of Agriculture, Forest Service; unpublished study; CDL:227336-K) MRID 00066098.

Mazzone HM; Tignor GH. 1976. Insect viruses: Serological relationships. Pages 237-270, In Advances in Virus Research: Volume 20. (Also In unpublished submission received Feb 22, 1980 under 27586-EX-25; submitted by U.S. Dept. of Agriculture, Forest Service, Washington, D.C.; CDL:241872-D) MRID 00030816.

Mazzone HM; Tignor GH; Shope RE; et al. 1976. A serological comparison of the nuclear polyhedrosis viruses of the gypsy moth and the European pine sawfly with arthropod-borne and other viruses. Environmental Entomology 5(2):281-282.

Mazzone HM; Breillatt J; Bahr G. 19??. Studies on the Rod Forms and Isolated Deoxyribonucleic Acid from the Nucleopolyhedrosis Virus of the Gypsy Moth (*Porthetria dispar* L.). (U.S. Forest Service, Oak Ridge National Laboratory and Armed Forces Institute of Pathology; unpublished study; CDL:231360-B) MRID 00068398.

McCarthy WJ; Liu SY. 19??. The Structural Proteins of *Porthetria dispar* Nuclear Polyhedrosis Virus. (Unpublished study received Aug 18, 1977 under 27582-2; prepared by Pennsylvania State Univ., Pesticide Research Laboratory and Graduate Study Center, submitted by Parker Livestock Supply, Inc., Fremont, Nebr.; CDL:231360-C) MRID 00068399.

Moore RB. 1977. Determination of the Effects of Nuclear Polyhedrosis Virus in Trout and Bluegill Sunfish under Laboratory Conditions. Cooperative Agreement No. 42-213.(Unpublished study received Aug 18, 1977 under 27582-2; prepared by Essex Marine Laboratory, Inc. and U.S. Forest Service, Northeastern Area, State and Private Forestry, submitted by Parker Livestock Supply, Inc. MRID 00054565.

Murray KD; Elkinton JS. 1989. Environmental contamination of egg masses as a major component of transgenerational transmission of gypsy moth nuclear polyhedrosis virus LdMNPV. J Invert Pathology. 53(3):324-334.

Murray KD; Elkinton JS. 1990. Transmission of nuclear polyhedrosis virus to gypsy moth Lepidoptera: Lymantriidae eggs via contaminated substrates. Environmental Entomology. 19(3):662-665.

Murray KD; Shields KS; Burand JP; Elkinton JS. 1991. The effect of gypsy moth metamorphosis on the development of nuclear polyhedrosis virus infection. Journal of Invertebrate Pathology 57:352-361.

Omnova Solutions. 1999. Material Safety Data Sheet for Carrier 038-A. OMNOVA Solutions Inc., Performance Chemicals, 6008 High Point Road, Greensboro, NC 27407. MSDS dated Nov. 19, 1999. Available at: http://www.dnr.state.wi.us/org/land/forestry/fh/GM/.

Onken A. 2004. Comments on peer review draft of Gypchek Risk Assessment. Email to Joseph Cook dated March 6, 2004.

Nealis, V.G.; Erb, S. 1993. A sourcebook for management of the gypsy moth. Sault Ste. Marie, Ontario: Forestry Canada, Ontario Region, Great Lakes Forestry Centre; 47 p. + app.

NIOSH (National Institute of Occupational Safety and Health). 2003. The Registry of Toxic Effects of Chemical Substances: 2,2'-stilbendisulfonic acid, 4,4'-bis((4-anilino-6-morpholino-s-triazin-2-yl)amino)-, disodium salt. www.cdc.gov/NIOSH/rtecs/wj5dcbb8.html.

Nutrilite Products Incorporated. 1967. Toxicity Study of Insect Viruses on Mammals and Humans. (Compilation; unpublished study received May 6, 1968 under 6G0481; CDL:090540-S) MRID 00082228.

Parker Livestock Supply Incorporated. 1975b. Persistence of *L. dispar* Nucleopolyhedrosis Virus following Oral Administration to Rats. (Unpublished study received Aug 18, 1977 under 27582-2; CDL:231360-J) MRID 00068405.

Parker Livestock Supply Incorporated. 19??a. Preparation of Production Innoculum from Gypsy Moth NPV Seed. (Unpublished study received Aug 18, 1977 under 27482-2; CDL:231360-A) MRID 00068397.

Parker Livestock Supply Incorporated. 19??c. Serological Evaluation of *L. dispar* NPV Acute Oral Toxicity Study. (Reference to Cannon Lab report 6E-2618; unpublished study received Aug 18, 1977 under 27582-2; CDL:231360-P) MRID 00068411.

Podgwaite JD. 1981. Natural disease within dense gypsy moth populations. In: The Gypsy Moth: Research Toward Integrated Pest Management. CC Doane and ML McManus eds. Forest Service. Science and Education Agency. Technical Bulletin 1584, p. 125-132.

Podgwaite JD. 1999. Gypchek: Biological insecticide for the gypsy moth. J Forestry. 97: 16-19.

Podgwaite JD; Bruen RB. 1978. Procedures for the Microbiological Examination of Production Batch Preparations of the Nuclear Polyhedrosis Virus (Baculovirus) of the Gypsy Moth, *Lymantria dispar* L. Broomall, Pa: U.S. Dept. of Agriculture, Forest Service, Northeastern Forest Exper (Forest Service general technical report NE-38; available from: U.S. Government Printing Office; published study; CDL:244763-O) MRID 00069824.

Podgwaite J; Galipeau P. 1978. Effect of Nucleopolyhedrosis Virus on Two Avian Predators of the Gypsy Moth. (Forest Service research note NE-251; also In unpublished submission received Sep 11, 1979 under 27586-2; submitted by U.S. Forest Service, Washington, DC; CDL:240994-I) MRID 00134318.

Podgwaite JD; Stone SK; Zerillo RT; Bruen RB. 1979. Environmental persistence of the nucleopolyhedrosis virus of the gypsy moth Lymantria-dispar. Environmental Entomology. 8(3):528-536.

Podgwaite JD; Reardon RC; Kolodny-Hirsch DM; Walton GS. 1991. Efficacy of ground application of the gypsy moth (Lepidoptera: lymantriidae) nucleopolyhedrosis virus product, Gypchek. J Econ Entomol. 84: 440-444.

Podgwaite JD; Reardon RC; Walton GS; Witcosky J. 1992a. Efficacy of aerially-applied Gypchek against gypsy moth (Lepidoptera: lymantriidae) in the Appalachian highlands. J Entomol Sci. 27: 337-344.

Podgwaite JD, Reardon RC, Walton GS; Venables L; Kolodny-Hirsch DM. 1992b. Effects of aerially applied Gypchek on gypsy moth (Lepidoptera: Lymantriidae) populations in Maryland woodlots. J Econ Entomol.85(4):1136-1139.

Podgwaite JD; Dubois NR; Reardon RC; Witcosky J. 1993. Retarding outbreak of low-density gypsy moth (Lepidoptera: lymantriidae) populations with aerial applications of Gypchek and *Bacillus thuringiensis*. J Econ Entomol. 86: 730-734.

Raimo B; Reardon R; Podgwaite J. 1977. Vectoring gypsy moth nuclear polyhedrosis virus by *Apanteles melanoscelus* Hym.: Braconidae. Entomophaga. 22(2): 207-215.

Rastall K; Kondo V; Strazanac JS; Butler L. 2003. Lethal effects of biological insecticide applications on nontarget lepidopterans in two Appalachian forests. Environ Entomol. 32(6): 1364-1369.

Reardon R; Podgwaite JD. 1992. The gypsy moth nucleopolyhedrosis virus product. USDA Forest Service, Northeastern Area, State and Private Forestry, Radnor, PA, NA-TP-02-92. 8 pp .

Reardon RC; Podgwaite JD. 1994. Summary of efficacy evaluations using aerially applied Gypchek moth in the U.S.A. J Environ Sci Health, Part B, Pestic Food Contam Agric Wastes. (4): 739-756.

Roberts S. 1978. Acute Oral Toxicity of LDP 53 (3.75 x 10^3 /g) in Bobwhite Quail. Laboratory No. 7E-9564. (Cannon Laboratories, Inc. for U.S. Forest Service; unpublished study; CDL: 232890-A) MRID 00091447.

Roberts RB; Pieper GR. 1976. Study Plan--Residue Analysis for Sevin-4-oil (Carbaryl), Orthene, and Dimilin in Cooperative Safety Tests on Non-target Organisms. (U.S. Forest Service, Pacific Southwest Forest and Range Experiment Station, Insecticide Evaluation Project; unpublished study; CDL:227609-F) MRID 00065694.

Roberts S; Wineholt RL. 1976. Report: 8-day Dietary Study of Gypsy Moth Virus in Mallard Ducks. Laboratory No. 6E-3281. (U.S. Forest Service, Northeastern Forest Excperiment Station, Forest Insect and Disease Laboratory; unpublished study; CDL: 231360-O) MRID 00068410.

Russell RL; Rohrmann GF 1997. Characterization of P91, A Protein Associated With Virions of An Orgyia Pseudotsugata Baculovirus. Virology. 233(1): 210-23.

Sandoz Incorporated. 1979. Efficacy of Elcar Heliothis Virus on Soybeans and Various Crops . (Reports by various sources; unpublished study including published data, received Jul 9, 1979 under 11273-17; CDL:243280-A) MRID 00046628.

SERA (Syracuse Environmental Research Associates, Inc.). 2001. Preparation of Environmental Documentation and Risk Assessments, SERA MD 2001-01a, draft dated July 2001. Syracuse Environmental Research Associates, Inc., Fayetteville, NY. Available at www.sera-inc.com.

SERA (Syracuse Environmental Research Associates, Inc.). 2003. Glossary of Environmental Terms, SERA TD 2003-03a, draft dated October 14, 2003. Syracuse Environmental Research Associates, Inc., Fayetteville, NY. Available at www.sera-inc.com.

Shapiro M; Bell RA. 1981. Biological activity of *Lymantria dispar* nucleopolyhedrosis virus from living and virus killed larvae. Annuals of the Entomological Society of America. 74(1):27-28.

Shapiro M; Robertson JL; Bell RA. 1986. Quantitative and qualitative differences in gypsy moth (Lepidoptera: Lymantriidae) nucleopolyhedrosis virus produced in different-aged larvae. Journal of Economic Entomology. 79(5):1174-1177.

Shieh TR; Bohmfalk GT. 1980. Production and efficacy of baculoviruses. Biotechnology and Bioengineering XXII:1357-1375. (Also in unpublished submission received Mar 6, 1981 under 11273-EX-23; submitted by Sandoz, Inc.--Crop Protection, San Diego, Calif.; CDL:099944-G) MRID 00072678.

Shope RE. 1976. Study of the Pathogenesis of the Nuclear Polyhedrosis Virus of the Gypsy Moth (*Porthetria dispar*) in Immunosuppressed Animals. (Yale Univ., Dept. of Epidemiology and Public Health, Yale Arbovirus Research Unit for U.S. Forest Service, Forest Insect and Disease Laboratory; unpublished study; CDL:230162-Y) MRID 00060710.

Shope RE; Tignor GH; Smith A; et al. 1975. Study of the Pathogenesis of the Nuclear Polyhedrosis Virus of the Gypsy Moth (*Porthetria dispar*) in Immuno Suppressed Animals. Interim rept. (Yale Univ., Dept. of Epidemiology and Public Health, Yale Arbovirus Research Unit for U.S. Forest Service, Forest Insect and Disease Laboratory; unpublished study; CDL:230162-L) MRID 00060700.

Slavicek JM; Podgwaite J; Lanner-Herrera C. 1992. Properties of two *Lymantria dispar* nuclear polyhedrosis virus isolates obtained from the microbial pesticide Gypchek. J Invertebr Pathol. 59: 142-148.

South Carolina Pest Management Program. 2001. Management of arthropods associated with production of vegetables and medicinal plants. Abstract available on-line at: www.clemson.edu/scg/pest/shepard3.htm

Streams FA. 1976. Susceptibility of Aquatic Invertebrates to Gypsy Moth NPV. Grant No. 23-636. Final rept. (Univ. of Connecticut, Biological Sciences Group, Ecology Section, U-42 for U.S. Forest Service; unpublished study; CDL:231360-M) MRID 00068408.

Terrell Y; Parke GSE. 1976a. Report: 30-Day Feeding Study of Gypsy Moth NPV (Lot # 33). Laboratory No. 6E-3600. (Unpublished study received Jan 11, 1977 under 27586-2; prepared by Cannon Laboratories, Inc., submitted by U.S. Dept. of Agriculture; CDL: 229738-A) MRID 00055915.

Terrell Y; Parke GSE. 1976b. Report: 30-Day Feeding Study of Gypsy Moth NPV: Laboratory No. 6E-3600. (Cannon Laboratories, Inc. for U.S. Forest Service, unpublished study; CDL:230813-A) MRID 00048862.

Terrell Y; Parke GSE. 1976c. Report on Acute I.P. Single Dose Toxicity in Mice. Laboratory No. 6E-2873. (Cannon Laboratories, Inc. for U.S. Forest Service, Insect & Disease Laboratory; unpublished study; CDL:227336-T) MRID 00066103.

Terrell Y; Parke GSE. 1976d. Report on Acute I.P. Single Dose Toxicity in Mice. Laboratory No. 6E-2874. (Cannon Laboratories, Inc. for U.S. Forest Service, Insect & Disease Laboratory; unpublished study; CDL:227336-AO) MRID 00066109.

Terrell Y; Parke GSE; Offenkrantz FM. 1976a. Report: 35-Day Feeding Study of Gypsy Moth NPV (Lot # 33). Laboratory No. 6E- 2618. (Unpublished study received Jan 11, 1977 under 27586-2; prepared by Cannon Laboratories, Inc., submitted by U.S. Dept. of Agriculture; CDL:229738-B) MRID 00055920.

Terrell Y; Parke GSE; Offenkrantz FM. 1976b. Report: 35-Day Feeding Study of Gypsy Moth NPV: Laboratory No. 6E-2618. (Cannon Laboratories, Inc. for U.S. Forest Service, unpublished study; CDL:230813-B) MRID 00048863.

Terrell Y; Parke GSE; Offenkrantz FM. 1976c. Report: 35-Day Feeding Study of Gypsy Moth NPV (Lot # 33). Laboratory No. 6E- 2618. (Cannon Laboratories, Inc. for U.S. Forest Service; unpublished study; CDL:230162-D) MRID 00060693.

Theilmann DA; Chantler JK; Stweart S; Flipsen HT; Vlak JM; Crook NE 1996. Characterization of A Highly Conserved Baculovirus Structural Protein That Is Specific for Occlusion-derived Virions. Virology. 218 (1):148-58.

Thornett HD. 1975. Final Report: Inhalation Toxicity Study-- Rats. Contract No. 12-14-110-4145-33; LBI Project No. 2241. (Litton Bionetics, Inc. for U.S. Dept. of Agriculture, Forest Service, NEFES, Forest Insect and Disease Laboratory, unpublished study; CDL:223573-G) MRID 00049266.

Thorpe KW; Podgwaite JD; Slavicek JM; Webb RE. 1998. Gypsy moth (Lepidoptera: lymantriidae) control with ground-based hydraulic applications of Gypchek, in vitro-produced virus, and bacillus thuringiensis. J Econ Entomol. 91: 875-880.

Thorpe KW; Cook SP; Webb RE; Podgwaite JD; Reardon RC. 1999. Aerial application of the viral enhancer Blankophor BBH with reduced rates of gypsy moth (Lepidoptera: lymantriidae) nucleopolyhedrovirus. Biol Control. 16: 209-216.

USDA (U.S. Department of Agriculture). 1995. Gypsy Moth Management in the United States: A Cooperative Approach. Final Environmental Impact Statement. Appendix F (Human Health Risk Assessment) and Appendix G (Ecological Risk Assessment).

USDA/FS (U.S. Department of Agriculture/Forest Service). 1963. Susceptibility of Insects to P. dispar NPV. (Unpublished study received Feb 22, 1980 under 27586-EX-25; CDL:241872-P) MRID 00030828.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1968?. Bioassay Protocol of NPV against Gypsy Moth Larvae. (Unpublished study; CDL:227336-L) MRID 00066099.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1973a. Efficacy of Nuclear-Polyhedrosis Virus for Gypsy Moths in Oak Trees. (Reports by various sources; unpublished study including published data, received on unknown date under 27586-EX-8; CDL:223573-I) MRID 00049268.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1973b. Chemical Characteristics of Nuclear Polyhedrosis Virus. (Compilation; unpublished study, including published data, received Dec 23, 1976 under 27586-2; CDL:227336-E) MRID 00066092.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1974. Gypsy Moth Virus Characteristics. (Compilation, unpublished study; CDL: 223573-A) MRID 00049260.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1975?. Persistance [sic] of *Lymantria dispar* Nucleopolyhedrosis Virus following Inhalation Exposure to Rats. (Unpublished study; CDL:232891-A) MRID 00090598.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1976b. Efficacy. (Compilation; unpublished study, including published data; CDL: 227336-AD) MRID 00066106.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1979a. Efficacy: Insect Viruses. (Compilation; unpublished study received Sep 11, 1979 under 27586-2; CDL:240994-B) MRID 00134312.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1979b. Efficacy: Nuclear Polyhedrosis Virus . (Compilation; unpublished study received Sep 11, 1979 under 27586-2; CDL:240994-J) MRID 00139935.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1997. Submission of Toxicity Data in Support of Reregistration for Gypchek. Transmittal of 2 Studies. MRID 44354300.

USDA/FS (U.S. Department of Agriculture/Forest Service). 19??a. General Product Chemistry. (Unpublished study; CDL:227336-A) MRID 00066089.

USDA/FS (U.S. Department of Agriculture/Forest Service). 19??b. Purity of Starting and Intermediate Materials. (Unpublished study; CDL: 227336-C) MRID 00066090.

USDA/FS (U.S. Department of Agriculture/Forest Service). 19??c. Complete Composition Including Impurities. (Unpublished study; CDL: 227336-J) MRID 00066097.

USDA/FS (U.S. Department of Agriculture/Forest Service). 19??d. Persistence of *L. dispar* Nucleopolyhedrosis Virus following Inhalation to Rats: Bioassay of Lung and Tracha. (Unpublished study; CDL:227336-Y) MRID 00066105.

USDA/FS (U.S. Department of Agriculture/Forest Service). 19??e. Environmental Chemistry. (Unpublished study; CDL:227336-M) MRID 00079586.

USDA/FS (U.S. Department of Agriculture/Forest Service). 19??f. Microbiological Evaluation. (Unpublished study; CDL:232891-C) MRID 00090600.

USDA/FS (U.S. Department of Agriculture/Forest Service). 19??g. Persistence of *L. dispar* Nucleopolyhedrosis Virus following Inhalation to Rats. (Unpublished study; CDL:230162-M) MRID 00060701.

USDA/FS (U.S. Department of Agriculture/Forest Service). 2002. B.t. Usage by State - 1999. www.fs.fed.us/na/morgantown/fhp/gmoth/gm_news47/chart1.htm.

USDA/FS (U.S. Department of Agriculture/Forest Service). 2003. Gypchek Product Label. Available at: http://www.dnr.state.wi.us/org/land/forestry/fh/GM/.

USDA/FS (U.S. Department of Agriculture/Forest Service). 2003. Gypchek Material Safety Data Sheet. Available at: http://www.dnr.state.wi.us/org/land/forestry/fh/GM/.

U.S. EPA 1996. Reregistration Eligibility Decision (RED): Polyhedral Inclusion Bodies of Gypsy Moth (*Lymantria dispar*) and Douglas Fir Tussock Moth (*Orgyia pseudotsugata*) Nuclear Polyhedrosis Viruses. EPA 738-R-96-020.

U.S. EPA (U.S. Environmental Protection Agency/Office of Research and Development). 1996. Exposure Factors Handbook. National Center for Environmental Assessment, U.S. EPA,

Washington, DC. EPA/600/P-95/002Ba-c. Avail. NTIS: PB97-117683, 97-117691, PB97-117709.

U.S. EPA/EFED (U.S. Environmental Protection Agency/Environmental Fate and Effects Division). 2001. Ecological Risk Assessor Orientation Package, Draft Version August 2001. Draft prepared by Brian Montague, Biologist, Ecological Fate and Effects Division, Office of Pesticide Programs.

Versoi PL; Yendol WG. 1982. Discrimination by the parasite, *Apanteles melanoscelus*, between healthy and virus-infected gypsy moth larvae. Environ Entomol. 11:42-45.

Wang C; Strazanac J; Butler L. 2000. Abundance, diversity, and activity of ants (Hymenoptera: formicidae) in oak-dominated mixed Appalachian forests treated with microbial pesticides. Environ Entomol. 29: 579-586.

Webb RE; Shapiro M; Podgwaite JD; Reardon RC; Tatman KM; Venables L; Kolodny-Hirsch DM. 1989. Effect of aerial spraying with Dimilin, Dipel, or Gypchek on two natural enemies of the gypsy moth (Lepidoptera: lymantriidae). J Econ Entomol. 82: 1695-1701.

Webb RE; Podgwaite JD; Shapiro M; Tatman KM; Douglass LW. 1990. Hydraulic spray application of Gypchek as a homeowner control tactic against gypsy moth (Lepidoptera: lymantriidae). J Entomol Sci. 25(3):383-393.

Webb RE; Shapiro M; Podgwaite JD; Lynn DE; Dougherty EM; Ridgway RL; Venables L; Cohen DL. 1993. Field comparison of different strains of gypsy moth nuclear polyhedrosis virus against gypsy moth (Lepidoptera: lymantriidae) in western Maryland in 1990. J Econ Entomol. 86: 1185-1190.

Webb RE; Shapiro M; Podgwaite JD; Ridgway RL; Venables L; White GB; Argauer RJ; Cohen DL; Witcosky J; Kester KM. 1994a. Effect of optical brighteners on the efficacy of gypsy moth (Lepidoptera: lymantriidae) nuclear polyhedrosis virus in forest plots with high or low levels of natural virus. J Econ Entomol. 87: 134-143.

Webb RE; Dill NH; Podgwaite JD; Shapiro M; Ridgway RL; Vaughn JL; Venables L; Argauer RJ. 1994b. Control of third and fourth instar gypsy moth (Lepidoptera: lymantriidae) with Gypchek combined with a stilbene disulfonic acid additive on individual shade trees. J Entomol Sci. 29: 82-91.

Webb RE; Peiffer RA; Fuester RW; Valenti MA; Thorpe KW; White GB; Shapiro M. 1999a. Effects of Blankophor BBH, a virus-enhancing adjuvant, on mortality of gypsy moth (Lepidoptera: lymantriidae). J Entomol Sci. 34: 391-403.

Webb RE; Thorpe KW; Podgwaite JD; Reardon RC; White GB; Talley SE. 1999b. Field evaluation of an improved formulation of Gypchek (a nuclear polyhedrosis virus product) against the gypsy moth (Lepidoptera: lymantriidae). J Entomol Sci. 34: 72-83.

Webb RE; Thorpe KW; Podgwaite JD; Reardon RC; White GB; Talley SE. 1999c. Efficacy of Gypchek against the gypsy moth (Lepidoptera: lymantriidae) and residual effects in the year following treatment. J Entomol Sci. 34: 404-414.

Woods SA; Elkinton JS. 1987. Bimodal patterns of mortality from nuclear polyhedrosis virus in gypsy moth (*Lymantria dispar*) populations. J Invert Pathol. 50:151-157.

Woods SA; Elkinton JS; Podgwaite JD. 1989. Acquisition of nuclear polyhedrosis virus from tree stems by newly emerged gypsy moth larvae. Environ Entomol. 18(2):298-301.

Woods SA; Elkinton JS; Shapiro M. 1990. Factors affecting the distribution of a nuclear polyhedrosis virus among gypsy moth (Lepidoptera: Lymantriidae) egg masses and larvae. Environ Entomol. 19(5):1330-1337.

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 40×10^9 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 1.8×10^{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 40×10^9 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 40×10^9 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 40×10^9 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 40×10^9 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 40×10^9 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" $(6.48 \times 10^{10}/g)$ (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 (3.73×10^{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; 6.6×10^{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 (= $4.04 \times 10^8 \pm 1.38 \times 10^8$ 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., 1.04×10^6, 5.2×10^6, 1.04×10^7, 1.04×10^8, 1.04×10^9 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 4th instar gypsy moth larvae on day 1 and on alternate days for 3 weeks. Each infected larva contained from 3.3×10^7 to 2.1×10^8 PIB. During the test period, each chickadee ate 70-80 infected larvae (from 2.3×10^9 to 1.7×10^{10} PIB) and each treated sparrow ate 90-100 infected larvae (from 3.0×10^9 to 2.1×10^{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 2.5×10^{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 2.5×10^{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 3-1

Appendix 4: Toxicity of NPV to Aquatic Invertebrates

Product	Species/Exposure	Observations	Reference
NPV containing 1.7×10^{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 1.7×10^{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* <u>Daphnia</u> *reared in water with a high concentration of polyhedra and , if so, how soon the NPV disappeared from* <u>Daphnia</u> *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 1.7×10^{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 4-1

Appendix 4: Toxicity of NPV to Aquatic Invertebrates			
Product	Species/Exposure	Observations	Reference
NPV containing 1.7×10^{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 1.7×10^{11} polyhedra/g and some bacterial impurities.	Water boatmen (adult *Hesperocorixa interrupta* [n=10/replicate] and *Sigara gordita* n=20/replicate]) exposed to NPV at a concentration in water of 250 polyhedra/mL for 4 weeks.	No significant difference in survival of either species in among treated and control adults and no apparent adverse effects on reproduction were observed in *Sigara*, which produced eggs, many of which hatched before the end of the study. Results of the bioassay indicate that the water boatmen did not accumulate NPV under the conditions of the study.	Streams 1976 MRID 00068408

Appendix 4-2

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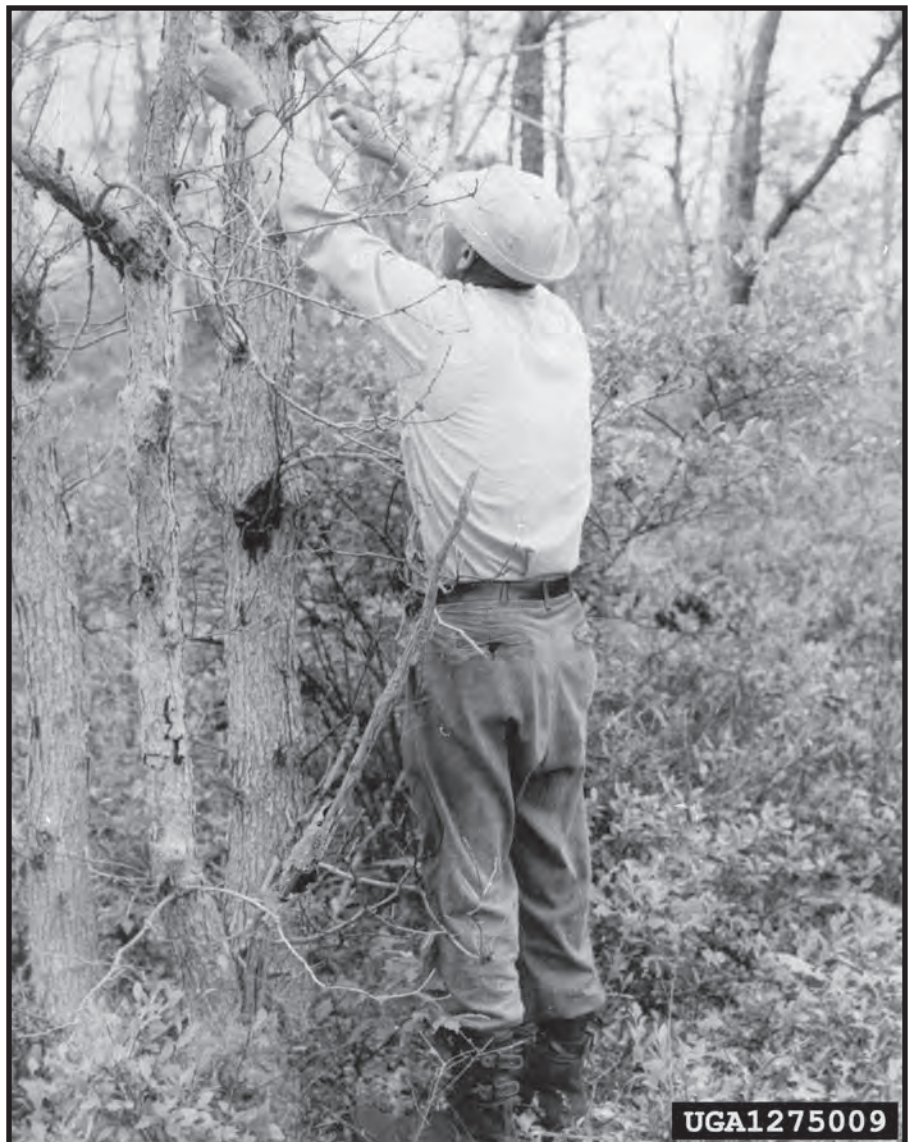
Figure H-1. Female gypsy moth pupae were gathered in Massachusetts in 1948 in order to obtain sex attractant for trapping programs.

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

PREFACE

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Appendix 3: Toxicity of disparlure aquatic species

Appendix 4: EPI Suite Output for Disparlure

LIST OF TABLES

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

Located at: http://www.fs.fed.us/foresthealth/pesticide/risk.shtml

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AEL	adverse-effect level
AGM	Asian Gypsy Moth
a.i.	active ingredient
BCF	bioconcentration factor
bw	body weight
CBI	confidential business information
cm	centimeter
CNS	central nervous system
EC_x	concentration causing X% inhibition of a process
EC_{25}	concentration causing 25% inhibition of a process
EC_{50}	concentration causing 50% inhibition of a process
F	female
FH	Forest Health
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FQPA	Food Quality Protection Act
g	gram
ha	hectare
HQ	hazard quotient
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
k_a	absorption coefficient
k_e	elimination coefficient
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
$K_{o/w}$	octanol-water partition coefficient
K_p	skin permeability coefficient
L	liter
lb	pound
LC_{50}	lethal concentration, 50% kill
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level
m	meter
M	male

mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
mM	millimole
MRID	Master Record Identification Number
MSDS	material safety data sheet
MW	molecular weight
NAGM	North American Gypsy Moth
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOS	not otherwise specified
NRC	National Research Council
NTP	National Toxicology Program
OPP	Office of Pesticide Programs
OPPTS	Office of Pesticide Planning and Toxic Substances
ppm	parts per million (used in expressing dietary concentrations only)
QSAR	quantitative structure activity relationship
RfD	reference dose
SERA	Syracuse Environmental Research Associates
SRC	Syracuse Research Corporation
UF	uncertainty factor
U.S.	United States
USDA	U.S. Department of Agriculture
U.S. EPA	U.S. Environmental Protection Agency
WHO	World Health Organization
μ	micron
$>$	greater than
$\geq$	greater than or equal to
$<$	less than
$\leq$	less than or equal to
$=$	equal to
$\simeq$	approximately equal to
$\sim$	approximately

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

To convert ...	Into ...	Multiply by ...
acres	hectares (ha)	0.4047
acres	square meters (m^2)	4,047
atmospheres	millimeters of mercury	760
centigrade	Fahrenheit	1.8 C + 32
centimeters	inches	0.3937
cubic meters (m^3)	liters (L)	1,000
Fahrenheit	centigrade	5/9 (F-32)
feet per second (ft/sec)	miles/hour (mi/hr)	0.6818
gallons (gal)	liters (L)	3.785
gallons per acre (gal/acre)	liters per hectare (L/ha)	9.34
grams (g)	ounces, (oz)	0.03527
grams (g)	pounds, (oz)	0.002205
hectares (ha)	acres	2.471
inches (in)	centimeters (cm)	2.540
kilograms (kg)	ounces, (oz)	35.274
kilograms (kg)	pounds, (lb)	2.2046
kilograms per hectare (hg/ha)	pounds per acre (lb/acre)	0.892
kilometers (km)	miles (mi)	0.6214
liters (L)	cubic centimeters (cm^3)	1,000
liters (L)	gallons (gal)	0.2642
liters (L)	ounces, fluid (oz)	33.814
miles (mi)	kilometers (km)	1.609
miles per hour (mi/hr)	cm/sec	44.70
milligrams (mg)	ounces (oz)	0.000035
meters (m)	feet	3.281
ounces (oz)	grams (g)	28.3495
ounces per acre (oz/acre)	grams per hectare (g/ha)	70.1
ounces per acre (oz/acre)	kilograms per hectare (kg/ha)	0.0701
ounces fluid	cubic centimeters (cm^3)	29.5735
pounds (lb)	grams (g)	453.6
pounds (lb)	kilograms (kg)	0.4536
pounds per acre (lb/acre)	kilograms per hectare (kg/ha)	1.121
pounds per acre (lb/acre)	mg/square meter (mg/m^2)	112.1
pounds per acre (lb/acre)	µg/square centimeter (µg/cm^2)	11.21
pounds per gallon (lb/gal)	grams per liter (g/L)	119.8
square centimeters (cm^2)	square inches (in^2)	0.155
square centimeters (cm^2)	square meters (m^2)	0.0001
square meters (m^2)	square centimeters (cm^2)	10,000
yards	meters	0.9144

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

CONVERSION OF SCIENTIFIC NOTATION

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

EXECUTIVE SUMMARY

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

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

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

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

HUMAN HEALTH RISK ASSESSMENT

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

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

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

Risk Characterization – Although studies on the acute toxicity of disparlure have been conducted in laboratory animals, the lack of subchronic or chronic toxicity data precludes a quantitative characterization of risk. The available data regarding the acute toxicity of disparlure indicate that the potential hazard from exposure to the compound is low.

The reliance on acute toxicity data introduces uncertainties into the risk assessment that cannot be quantified. Other uncertainties in this analysis are associated with the exposure assessment and involve environmental transport and dermal absorption. These uncertainties are relatively minor compared to the lack of subchronic or chronic toxicity data. Thus, while there is no reason to believe that longer-term exposure to disparlure will produce adverse effects, this assumption can not be substantiated due to the lack of chronic toxicity data. The significance of this uncertainty is at least partially offset by the very low exposures that are plausible given the low application rates and the nature of plausible exposures of humans to disparlure.

ECOLOGICAL RISK ASSESSMENT

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

Results of acute gavage and dietary toxicity studies in mallard ducks and bobwhite quail show that disparlure has very low toxicity in these species, with no mortalities observed following exposure to up to 2510 mg/kg bw in bobwhite quail.

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

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

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

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

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

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

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

1. INTRODUCTION

The USDA Forest Service uses disparlure and the formulation of disparlure as Disrupt II in programs to control or eradicate gypsy moth populations. This document is an update to a risk assessment prepared in 1995 (USDA 1995) and provides risk assessments for human-health effects and ecological effects to support an assessment of the environmental consequences of these uses.

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

Although this is a technical support document and addresses some specialized technical areas, an effort was made to ensure that the document can be understood by individuals who do not have specialized training in the chemical and biological sciences. Certain technical concepts, methods, and terms common to all parts of the risk assessment are described in plain language in a separate document (SERA 2006).

The human health and ecological risk assessments presented in this document are not, and are not intended to be, comprehensive summaries of all of the available information. No published reviews regarding human health or ecological effects of disparlure have been encountered. Moreover, almost all of the mammalian toxicology studies and most of the ecotoxicology studies are unpublished reports submitted to the U.S. EPA as part of the registration process for disparlure.

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

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

2. PROGRAM DESCRIPTION

2.1. OVERVIEW

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

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

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

2.2. CHEMICAL DESCRIPTION

Disparlure is the common name for cis-7,8-epoxy-2-methyloctadecane:
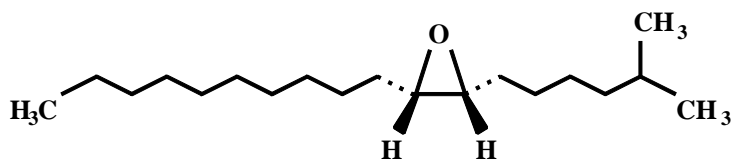
Disparlure can take two enantiomer forms, referred to as (+)disparlure and (-)disparlure. The term *enantiomer* refers to molecules that are structurally identical except for differences in the 3-dimensional configuration such that one form is the mirror image of the other.

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

(Schaefer et al. 1999). As with the gypsy moth, both of these *Lymantria* species are forest pests and adverse effects on these species are not a substantial concern for this risk assessment.

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

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

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

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

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

2.3. APPLICATION METHODS AND RATES

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

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

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

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

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

2.4. USE STATISTICS

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

3. HUMAN HEALTH RISK ASSESSMENT

3.1 HAZARD IDENTIFICATION

3.1.1 Overview.

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

Results of acute toxicity studies for oral, dermal, ocular and inhalation exposure to disparlure are summarized in Table 3-1. With the exception of one acute gavage study in rats using the 50:50 racemic mix (Coleman 2000), none of the toxicity studies specified whether the 50:50 racemic mix or the (+)enantiomer was tested. Based on the results of studies on disparlure, acute exposure to disparlure appears to pose a very low risk to mammals. No studies investigating the effects of chronic exposure of mammals to disparlure or studies investigating the effects of disparlure on the nervous system, immune system, reproductive system or endocrine system were identified. The carcinogenic potential of disparlure has not been assessed. The results of a single study show that disparlure is not mutagenic.

3.1.2 Mechanism of Action

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

3.1.3 Kinetics and Metabolism

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

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

3.1.4 Acute Oral Toxicity

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

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

3.1.5 Subchronic and Chronic Systemic Toxic Effects

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

3.1.6 Effects on Nervous System

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

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

3.1.7 Effects on Immune System

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

3.1.8 Effects on Endocrine System

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

3.1.9. Reproductive and Teratogenic Effects

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

3.1.10. Carcinogenicity and Mutagenicity

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

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

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

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

3.1.12. Systemic Toxic Effects from Dermal Exposure

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

3.1.13. Inhalation Exposure

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

3.1.14. Inerts and Adjuvants

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

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

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

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

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

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

3.1.15. Impurities and Metabolites

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

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

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

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview

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

3.2.2. Dermal Exposure

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

3.2.3. Inhalation Exposure

Both workers and the public may be exposed to disparlure by inhalation and the magnitude of the exposure can be estimated from available monitoring studies. In these studies, high application rates, relative to the projected rates used in program activities (29.1 g/acre, Section 2.3), were used in order to be able to detect disparlure in air.

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

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

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

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

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

3.2.4. Oral Exposure

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

3.3. DOSE-RESPONSE ASSESSMENT

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

$$\text{Oral LD}_{50} > 34{,}600 \text{ mg/kg}$$
$$\text{Dermal LD}_{50} > 2{,}025 \text{ mg/kg}$$
$$\text{Inhalation LC}_{50} > 5 \text{ mg/L x 1 hour}$$

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

3.4. RISK CHARACTERIZATION

3.4.1 Overview

Although studies on the acute toxicity of disparlure have been conducted in laboratory animals, the lack of subchronic or chronic toxicity data precludes a quantitative assessment of risk for longer-term exposures. The available data regarding the acute toxicity of disparlure indicate that the potential hazard from exposure to the compound is low.

The reliance on acute toxicity data introduces uncertainties into the risk assessment that cannot be quantified. Other uncertainties in this analysis are associated with the exposure assessment and involve environmental transport and dermal absorption. Thus, while there is no reason to believe that longer-term exposure to disparlure will produce adverse effects, this assumption can not be substantiated due to the lack of chronic toxicity data. The significance of this uncertainty is at least partially offset by the very low exposures that are plausible given the low doses of disparlure used in programs to control the gypsy moth.

3.4.2. Workers and the General Public

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

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

3.4.3. Sensitive Subgroups

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

3.4.4. Cumulative Effects

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

3.4.5. Connected Actions

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

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview

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

Results of acute gavage and dietary toxicity studies in mallard ducks and bobwhite quail show that disparlure has very low toxicity in these species, with no mortalities observed following exposure to up to 2510 mg/kg bw in bobwhite quail.

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

4.1.2. Toxicity to Terrestrial Organisms

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

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

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

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

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

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

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

4.1.3. Aquatic Organisms

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4.2. EXPOSURE ASSESSMENT

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

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

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

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

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

As discussed in Section 2.3, this risk assessment considers application rates in the range of 6 grams a.i./acre to 15 grams a.i./acre. This range corresponds to application rates of about 1.5 mg/m^2 [6 grams a.i./acre $\times$ 1000 mg/g $\times$ 1 acre/4047 m^2 = 1.4826 mg/m^2] to 3.7 mg/m^2 [15 grams a.i./acre $\times$ 1000 mg/g $\times$ 1 acre/4047 m^2 = 3.7064 mg/m^2]. If these amounts of disparlure are applied accidentally to a 1 meter deep body of water, nominal concentrations – i.e., assuming complete mixing and ignoring solubility limitations – would be in the range of 0.0015 mg/L to 0.0037 mg/L [1000 liters per m^3]. Details of these calculations are given in Worksheet A01 of the EXCEL workbook that accompanies this risk assessment.

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

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

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

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1 Overview

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

4.3.2. Fish

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

4.3.3. Aquatic invertebrates

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

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

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

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

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

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

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

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

4.4. RISK CHARACTERIZATION

4.4.1. Overview

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

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

4.4.2. Terrestrial Species

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

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

4.4.3. Fish

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

4.4.4. Aquatic Invertebrates

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

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

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

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

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

5. REFERENCES

Appleton H. 2006. Toxicologist, USDA Forest Service, Forest Health Protection. Washington, D.C. Email: happleton@fs.fed.us. Personal communication via telephone with Patrick Durkin (SERA, Inc) on June 29, 2006.

Beroza M; Inscoe MN; Schwartz PH; Keplinger ML; Mastri CW. 1975a. Acute toxicity studies with insect attractants. Toxicology and Applied Pharmacology 31: 421.

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

Bierl BA. 1977?. Rate of emission of disparlure from laminated plastic dispensers as affected by temperature and air flow rate. (U.S. Dept. of Agriculture, Science and Education Administration, Agricultural Environmental Quality Institute, unpublished study; CDL:236537-E). MRID 00047219

Bierl-Leonhardt BA; DeVilbiss ED; Plimmer JR. 1979. Rate of release of disparlure form laminated plastic dispensers. J Econ Entomol 72:319-321.

Cameron EA. 1981. On the persistence of disparlure in the human body. Journal of Chemical Ecology 7(2): 313-318.

Cameron EA. 1983. Apparent long-term bodily contamination by disparlure, the gypsy moth (*Lymantria dispar*) attractant. Journal of Chemical Ecology 9(1): 33-37.

Cameron EA. 1995. On the apparent persistence of disparlure in the human body. J Chem Ecol 21(4):385-386.

Campbell RW. 1983. Gypsy moth (Lepidoptera: Lymantriidae) control trials combining nucleopolyhedrosis virus, disparlure, and mechanical methods. Journal of Economic Entomology 76(3): 610.

Caro JH; Bierl BA; Freeman HP; Glotfelty DE; Turner BC. 1977. Disparlure: Volatilization rates of two microencapsulated formulations from a grass field. Environ Entom 6(6):877-881.

Caro JH; Freeman HP; Brower DL; Bierl-Leonhardt BA. 1981. Comparative distribution and persistence of disparlure in woodland air after aerial application of three controlled-released formulations (*Lymantria dispar*, gypsy moth sex pheromone). Journal of Chemical Ecology 7(5): 867-880.

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

Coleman D. 2000. Racemic disparlure Acute Oral Toxicity to the Rat (Acute Toxic Class Method). (Disparlure Technical): Lab Project Number: SHE 032/003649/AC. Unpublished study prepared by Huntingdon Life Sciences Limited. 15 p. {OPPTS 870.1100} MRID 45529801

Dourson ML; Stara JF. 1983. Regulatory history and experimental support for uncertainty (safety) factors. Regulatory Toxicology and Pharmacology 3: 224-238.

Durkin PR; Diamond G. 2002. Neurotoxicity, Immunotoxicity, and Endocrine Disruption with Specific Commentary on Glyphosate, Triclopyr, and Hexazinone: Final Report. SERA TR 01-43-08-04a dated January 30, 2002. Available at www.fs.fed.us/foresthealth/pesticide/risk.htm.

EPI Suite. 2006. Estimates of Chemical and Physical Properties of Disparlure Based on EPI Suite Version 3.12. EPI Suite runs conducted by Patrick Durkin, SERA, Inc. on June 28, 2006. Copy of EPI Suite run include with this risk Assessment as Appendix 4.

Fink R; Beavers JB; Joiner G; et al. 1980. Final Report: Acute Oral LD50--Bobwhite Quail: Project No. 173-102. (Unpublished study received Nov 19, 1980 under 36638-5; prepared by Wildlife International, Ltd., submitted by Conrel, an Albany International Co., Needham Heights, Mass.; CDL:244729-A). MRID 00083102

French-McCay DP. 2002. Development and Application of an Oil Toxicity and Exposure Model, OilToxEx. Environmental Toxicology and Chemistry. 21(10): 2080–2094.

Grapenthien JR. 1972. Report to United States Department of Agriculture: Acute Aerosol Inhalation Toxicity Study with Dispar- lure in Albino Rats: IBT No. A1958. (Unpublished study received Feb 21, 1980 under 36638-5; prepared by Industrial Bio-Test Lab- oratories, Inc., submitted by Conrel, an Albany International Co., Needham Heights, Mass.; CDL:242022-H). MRID 00059821

Gries G; Schaefer PW; Gries R; Liska J; Gotoh T. 2001. Reproductive character displacement in *Lymantria monacha* from northern japan? J Chem Ecol. 27(6): 1163-76.

Hercon Environmental. 1993. Letter from Priscilla MacLean (Hercon) to Noel Schneeberger dated June 22, 1993. Includes Product Label and Material Safety Data Sheet for Disrupt II.

Hercon Environmental. 2004. Hercon Disrupt II Fact Sheet. Copy courtesy of Donna Leonard, USDA Forest Service, Forest Health Protection, PO Box 2680, Asheville, NC 28802. e-mail: dleonard@fs.fed.us. Received July 19, 2004.

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

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

Herculite Products Incorporated. 1978. Hercon Luretape with disparlure: Gypsy Moth Pheromone Dispensers. (Reports by various sources; unpublished study including published data, received Dec 1, 1978 under 8730-17; CDL:236537-L). MRID 00047223

Hudson RH. 1975. Report on the Study To Determine the LC_{50} of disparlure for Mallards and Bobwhite Quail. (U.S. Fish and Wildlife Service, unpublished study; CDL:236537-N). MRID 00047225

Jacobson, M. 1976. Impact of natural plant protectants on the environment. In: Marini-Bettolo, G.B., ed. Natural products and the protection of plants: proceedings of a study week at the Pontifical Academy of Sciences, Oct. 18-23, 1976. Amsterdam: Elsevier Scientific Publishing Company; 22 p.

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

Kolodny-Hirsch DM; Webb RE. 1993. Mating disruption of gypsy moth Lepidoptera Lymantriidae following ground application of high rates of racemic disparlure. Journal of Economic Entomology 86(3): 815-820.

Knapp T; Terrell Y. 1980. Static 96-hour Toxicity Study of Neat Gypsy Moth Pheromone in Bluegill Sunfish: Laboratory No. OF- 7473. (Unpublished study received Nov 19, 1980 under 36638-5; prepared by Cannon Laboratories, Inc. submitted by Albany International, Controlled Release Div., Needham Heights, MA; CDL:244731-A). MRID 00127869

Kretchmar B. 1972. Report to United States Department of Agriculture: Acute Toxicity Studies with disparlure: IBT No. A1958. (Unpublished study received Nov 21, 1972 under 19750-1; prepared by Industrial Bio-Test Laboratories, Inc., submitted by American Can Co., Rahway, NJ; CDL:004647-B). MRID 00140660

LeBlanc G; Surprenant D; Sleight B; et al. 1980. Acute Toxicity of a Gypsy Moth Mating Disruption Pheromone, Active Ingredient Cis-7, 8-epoxy-2-octadene to the Water Flea : Report #BW-80-8-715. (Unpublished study received Nov 19, 1980 under 36638-5; prepared by EG & G, Bionomics, submitted by Albany International, Controlled Release Div., Needham Heights, MA; CDL:244730-A). MRID 00127868.

Leonard D. 2004. Comments on SERA TR 04-43-05-04a, Control/Eradication Agents for the Gypsy Moth – Human Health and Ecological Risk Assessment for Disparlure – Peer Review Draft. Comments by Donna Leonard, USDA Forest Service, Forest Health Protection, Asheville, NC. Comments received via email from dleonard@fs.fed.us.

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

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

Leonard D. 2006c. Field Observations in Applications of Disparlure. Comments by Donna Leonard, USDA Forest Service, Forest Health Protection, Asheville, NC. Comments received via email from dleonard@fs.fed.us on June 29, 2006.

Leonard D. 2006d. Review of SERA TR 06-52-02-01a, Control/Eradication Agents for the Gypsy Moth - Human Health and Ecological Risk Assessment for Disparlure (a.i.) and Disrupt II formulation – REVISED FINAL REPORT. USDA Forest Service, Forest Health Protection, Asheville, NC. Comments received via email from dleonard@fs.fed.us on July 12, 2006.

Leonard D. 2006e. Comments on Disrupt formulations by Donna Leonard, USDA Forest Service, Forest Health Protection, Asheville, NC. Comments received via email from dleonard@fs.fed.us on August 24, 2006.

Leonhardt BA; Mastro VC; Paszek EC; Schwable CP.; DeVilbiss ED. 1990. Dependence of gypsy moth (Lepidoptera: Lymantriidae) capture on pheromone release rate form laminate and other dispensers. J Econ Entomol. 83(5):1977-1981.

Leonhardt BA; Mastro VC; Devilbiss ED. 1993. New dispenser for the pheromone of the gypsy moth Lepidoptera Lymantriidae. Journal of Economic Entomology 86(3): 821-827.

Leonhardt BA; Mastro VC; Leonard DS; McLane W; Reardon RC; Thrope DS. 1996. Control of low-density gypsy moth (Lepidoptera: Lymantriidae) populations by mating disruption with pheromone. J Chem Ecology 22:1255-1272.

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

Meylan W; Howard P. 2000. Estimation Program Interface, Version 3.12. Syracuse Research Corporation, Syracuse, N.Y. for U.S. Environmental Protection Agency, Office of Pollution, Prevention and Toxics, Washington D.C. Downloadable copy of EPI-SUITE computer program available at: http://www.epa.gov/opptintr/exposure/docs/episuite.htm

Moody DE; Montgomery KA; Ashour MB; Hammock BD. 1991. Effects of environmentally encountered epoxides on mouse liver epoxide-metabolizing enzymes. Biochem Pharmacol 1991 Jun 1;41(11):1625-37.

Morewood P; Gries G; Haubler D; Moller K; Liska J; Kapitola P; Bogenschutz H. 1999. Towards pheromone-based detection of *Lymantria monarcha* (Lepidoptera: Lymanstriidae) in North America. The Canadian Entomogolsit 131:687-694.

Morewood P; Gries G; Haubler D; Moller K; Liska J; Kapitola P; Bogenschutz H. 2000. Towards pheromone-based monitoring of nun moth, *Lymantria monacha* (L.) (Lep., Lymantriidae) populations. J Appl Ent 124:77-85.

MTM Chemicals. 1991. Disparlure Material Safety Data Sheet. Prepared by MTM Chemicals, Inc. 1970 Atlas Street, Columbus, OH.

Murlis J; Willis MA; Carde RT. 2000. Spatial and temporal structures of pheromone plumes in fields and forests. Physiol Entomol 25:211-222.

NRC (National Research Council). 1983. Risk assessment in the Federal government: managing the process. Washington, DC: National Academy Press; 176 p. + app.

Oguma Y. 1998. Mutagenicity Testing of 7,8-Epoxy-2-Methyloctadecane in Bacterial Reverse Mutation Assays: Lab Project Number: 6128. Unpublished study prepared by BML, Inc. 19 p. {OPPTS 870.5265}. MRID 45309502

Oguma Y. 2000. Product Chemistry of Gypsy Moth Pheromone. (disparlure). Unpublished study prepared by Shin-Etsu Chemical Company. 22 p. MRID 45309501

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

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

Pelletier MC; Burgess RM; Ho KT; Kuhn; McKinney RA; Ryba SA. 1997. Phototoxicity of Individual Polycyclic Aromatic Hydrocarbons and Petroleum to Marine Invertebrate Larvae and Juveniles. Environmental Toxicology and Chemistry. 16(10): 2190-2199.

Plettner E; Lazar J; Prestwich EG; Prestwich GD. 2000. Discrimination of pH enantiomers by two pH binding proteins from the gypsy moth *Lymantria dispar*. Biochemistry 2000 Aug 1;39(30):8953-62.

Plimmer JR; Schwalbe CP; Paszek EC; et al. 1977. Contrasting effectiveness of (+) and (-) enantiomers of disparlure for trapping native populations of gypsy moth in Massachusetts. Environmental Entomology 6(4):518-522. (Also~In~unpublished submission received Aug 12, 1981 under 8730-31; submitted by Herculite Products, Inc., New York, N.Y.; CDL:245766-D). MRID 00080044

Rausina G. No Date. Report to United States Department of Agriculture, Agricultural Research Service, Agricultural Environmental Quality Institute: Results of Four-Day Static Fish Toxicity Studies: Rainbow Trout and Bluegills: IBT No. A-1958. (Unpublished study received Jan 22, 1975 under 11312-7; prepared by Industrial Bio-Test Laboratories, Inc., submitted by U.S. Dept. of Agriculture, Washington, D.C.; CDL:228392-B). MRID 00059735. *[**Note**: This study is catalogued by the U.S. EPA with a date of 1949 but disparlure had not been identified in 1949. The 1949 date used by U.S. EPA appears to be associated with a citation to statistical methods used by Rausina rather than the date of the study. The submission by Rausina does not indicate the date for the conduct of the study.]*

Schaefer PW; Gries G; Gries R; Holden D. 1999. Pheromone components and diel periodicity of pheromonal communication in *Lymantria funida*. J Chem Ent 25(10):2305-2312.

Schwalbe C; Paszek E; Webb R; et al. 1978. Field Evaluation of Controlled Release Formulations of disparlure for Gypsy Moth Mating Disruption. (Unpublished study received Apr 27, 1979 under 36638-EX-2; prepared by U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service, Gypsy Moth Methods Development Center and Others, submitted by Albany International, Controlled Release Div.,

Schwalbe C; Paszek E; Webb R; Bierl-Leionhardt BA; Plimmer JR; McComb CW; Dull CW. 1979. Field evaluation of controlled release formulations of disparlure for gypsy moth mating disruption. J Econ Entomol 72:322-236.

SERA (Syracuse Environmental Research Associates, Inc.). 2004. Control/Eradication Agents for the Gypsy Moth - Human Health and Ecological Risk Assessment for Disparlure (a.i.) - FINAL REPORT, SERA TR 04-43-05-04b, reported dated August 27, 2004.   Syracuse Environmental Research Associates, Inc., Fayetteville, NY.

SERA (Syracuse Environmental Research Associates, Inc.). 2006. Preparation of Environmental Documentation and Risk Assessments, SERA MD 2006-01a, draft dated March 3, 2006. Syracuse Environmental Research Associates, Inc., Fayetteville, NY. Available at www.sera-inc.com

Shin-Etsu Chemical Co Ltd. 2002. Submission of Product Chemistry Data in Support of the Application for Registration of disparlure Technical.   MRID 45810500

Taylor AW. 1982. Field measurements of pheromone vapor distribution. In: Leonhardt, B.A.; Beroza, M., eds. Insect pheromone technology: chemistry and applications. Washington, DC: American Chemical Society. ACS Symposium Series 190; 193-207.

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

Thorpe WE; Ridgway RL; Leonhardt BA. 1993. Relationship between gypsy moth Lepidoptera Lymantriidae pheromone trap catch and population density comparison of traps baited with 1 and 500 Micro Dextro disparlure lures. Journal of Economic Entomology 86(1): 86-92.

Thorpe WE; Leonhardt BA; Mastro VC; Reardon RC; Sellers P; Webb RE; Talley SE. 1999. Effectiveness of gypsy moth mating disruption from aerial applications of plastic laminate flakes with and without a sticking agent. TEKTRAN, www.nal.usda.gov/ttic/tektran/data/000010/74/0000107476.html.

Tobin PC; Leonard DS. 2006. Estimating Pheromone Released by Female Gypsy Moths During an Outbreak and Comparing this with Racemic Disparlure Released after an Application of Disrupt II. Unpublished analysis dated August 2, 2006. Copy courtesy of Donna Leonard, USDA Forest Service, Forest Health Protection, PO Box 2680, Asheville, NC 28802. e-mail: dleonard@fs.fed.us. Received August 7, 2006.

USDA (U.S. Department of Agriculture). 1995. Gypsy Moth Management in the United States: A Cooperative Approach. Final Environmental Impact Statement. Appendix F (Human Health Risk Assessment) and Appendix G (Risk Assessment).

USDA/FS (U.S. Department of Agriculture/Forest Service). 2005. Gypsy Moth Digest. Available at: http://na.fs.fed.us/fhp/gm/index.shtm

U.S. EPA (U.S. Environmental Protection Agency). 1994. Arthropod pheromones in solid matrix dispensers; experimental use permits. Federal Register 59(17): 3681-3684.

U.S. EPA (U.S. Environmental Protection Agency). 1996. Ecological Effects Test Guidelines OPPTS 850.1010: Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids. EPA 712–C–96–114 dated April 1996. U.S. EPA Office of Prevention, Pesticides and Toxic Substances. Available at: http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/850_Ecological_ Effects_Test_Guidelines/Drafts/850-1010.pdf.

U.S. EPA (U.S. Environmental Protection Agency). 2003. Toxicity Categories and Pesticide Label Statements. Available at: http://www.epa.gov/pesticides/health/tox_categories.htm

U.S. EPA/OPPTS (U.S. Environmental Protection Agency/Office of Prevention, Pesticides, and Toxic Substances). 1996. Ecological Effects Test Guidelines. OPPTS 850.1075: Fish Acute Toxicity Test, Freshwater and Marine. Available at: http://www.epa.gov/opptsfrs/home/guidelin.htm

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

U.S. EPA/OPPTS (U.S. Environmental Protection Agency/Office of Prevention, Pesticides, and Toxic Substances). 2006. Harmonized Test Guidelines. Available at http://www.epa.gov/opptsfrs/home/guidelin.htm

Ward GS. 1981. Acute Toxicity of a Synthetic Gypsy Moth Pheromone to Eastern Oysters (*Crassostrea virginica*): Report No. BP-81-3-31. (Unpublished study received Apr 17, 1981 under 36638-5; prepared by EG & G Bionomics, submitted by Conrel, an Albany International Co., Needham Heights, Mass.; CDL:244882-A). MRID 00074291

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

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

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

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

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

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

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

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

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

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

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

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

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

LIST OF APPENDICES

Appendix 1: Acute toxicity of disparlure to experimental mammals

Appendix 2: Toxicity of disparlure to birds

Appendix 3: Toxicity of disparlure aquatic species

Appendix 4: EPI Suite Output for Disparlure

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

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

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

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

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

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

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

Animal	Dose/Exposure	Response	Reference
FISH			
Rainbow trout Bluegills, 10 fish per concentration	0.1, 1.0, 10.0, 100.0 ppm (mg a.i./L) for 96 hours. Survival assessed at 1-6, 24, 48, 72, and 96 hours. Note: Very poor quality fiche. Dissolved oxygen was measured in the test water only when mortality was observed. The measurement itself cannot be read from the fiche.	No effect on dissolved oxygen. In bluegills, no affect on survivors at any concentration up to 96 hr exposure. **LC$_{50}$>100 ppm** In Rainbow trout, for all concentrations, no affect on survivors up to 48 hours. At the 100 ppm concentration, the number of survivors decreased to 8/10 after 72 hours of exposure. **LC$_{50}$>100 ppm**	Rausina No Date MRID 00059735

Bluegill sunfish, 30 fish in each group	Nominal concentration of 0 ppm (untreated control) and 300 ppm for 96 hours. No aeration during the study.
	
	No description of how the test water was prepared. No discussion of any observations concerning a surface film on the water.
		No mortalities observed and no signs of altered behavior.
		
		Dissolved oxygen in test water and control water were comparable:
		Day 1 11.0 ppm (control)
		  10.4 ppm (test water)
		Day 4: 3.4 ppm (control)
		  3.4 ppm (test water)
		pH constant in test and control water (pH 6.4) of the duration of testing.
		
		LC$_{50}$>300 ppm
			Knapp and Terrell 1980 MRID 00127869

AQUATIC INVERTEBRATES			

Technical Grade Disparlure

Animal	Dose/Exposure	Response	Reference
Eastern oysters (*Crassostrea virginica*)	96 hour exposure to concentrations ranging from 1.25 to 20 ppm 92% disparlure Acetone concentrations ranged up to 10%	No affect on new shell growth at any concentration **NOEC > 20 ppm**	Ward 1981 MRID 00074291

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

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

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

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

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

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

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

Appendix 3-3

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

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

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

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

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

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

Appendix 4: EPI Suite Output for Disparlure
      Run conducted on June 28, 2006 by Patrick Durkin using EPI-Suite Version 3.12.

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

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

                Log Kow(version 1.67 estimate): 8.08

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



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

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


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

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

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

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


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


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



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

          Water Sol: 0.001939 mg/L

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

Appendix 4-2

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

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

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


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

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

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

```
ECOSAR Program (v0.99h) Results:
==============================
SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
CAS Num:
ChemID1:
ChemID2:
ChemID3:
MOL FOR: C19 H38 O1
MOL WT : 282.51
Log Kow: 8.08  (KowWin estimate)
Melt Pt:
Wat Sol: 0.0007897 mg/L  (calculated)
ECOSAR v0.99h Class(es) Found
------------------------------
Epoxides

                                                            Predicted
ECOSAR Class                   Organism           Duration  End Pt  mg/L
(ppm)
=========================  ==================  ========  ======
==========
Neutral Organic SAR        : Fish               14-day    LC50    0.00192
*
(Baseline Toxicity)

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


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

       Bond Est :  1.49E-002 atm-m3/mole
       Group Est:  6.14E-002 atm-m3/mole

SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
MOL FOR: C19 H38 O1
MOL WT : 282.51
------------------------- HENRYWIN v3.10 Results -------------------------
----------+-----------------------------------------------+---------+---------
   CLASS  |      BOND CONTRIBUTION DESCRIPTION             | COMMENT | VALUE
----------+-----------------------------------------------+---------+---------
 HYDROGEN |  38  Hydrogen to Carbon (aliphatic) Bonds      |         | -4.5477
```

Appendix 4-4

```
FRAGMENT |  18  C-C                                        |         | 2.0935
FRAGMENT |   2  C-O                                        |         | 2.1709
FACTOR   |   *  Epoxide                                    |         |  .5000
---------+------------------------------------------------+---------+----------
RESULT   |    BOND ESTIMATION METHOD for LWAPC VALUE       | TOTAL   | 0.217
---------+------------------------------------------------+---------+----------
```

HENRYs LAW CONSTANT at 25 deg C = 1.49E-002 atm-m3/mole
                                 = 6.07E-001 unitless

```
--------+------------------------------------------------+----------+-------
        |         GROUP CONTRIBUTION DESCRIPTION          | COMMENT  | VALUE
--------+------------------------------------------------+----------+-------
        |        3   CH3 (X)                              |          | -1.86
        |       13   CH2 (C)(C)                           |          | -1.95
        |        1   CH (C)(C)(C)                         |          |  0.24
        |        2   CH (C)(C)(O)                         |          |  0.24
        |        1   O (C)(C)                             |          |  2.93
--------+------------------------------------------------+----------+-------
 RESULT | GROUP ESTIMATION METHOD for LOG GAMMA VALUE     |  TOTAL   | -0.40
--------+------------------------------------------------+----------+-------
```

HENRYs LAW CONSTANT at 25 deg C = 6.14E-002 atm-m3/mole
                                 = 2.51E+000 unitless

Henrys LC [VP/WSol estimate using EPI values]:
    HLC: 6.556E-002 atm-m3/mole
    VP: 0.000342 mm Hg
    WS: 0.00194 mg/L

BIOWIN (v4.02) Program Results:
==============================
SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
MOL FOR: C19 H38 O1
MOL WT : 282.51
------------------------- BIOWIN v4.02 Results --------------------------

   Biowin1 (Linear Model Prediction)   : Does Not Biodegrade Fast
   Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast
   Biowin3 (Ultimate Biodegradation Timeframe): Weeks
   Biowin4 (Primary Biodegradation Timeframe): Days-Weeks
   Biowin5 (MITI Linear Model Prediction)   : Does Not Biodegrade Fast
   Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast
   Ready Biodegradability Prediction: NO

------+-----+---+---------+--------
TYPE | NUM | Biowin1 FRAGMENT DESCRIPTION | COEFF | VALUE
------+-----+---+---------+--------
 Frag | 1 | Linear C4 terminal chain [CCC-CH3] | 0.1084 | 0.1084
```

```
Frag | 1 | Aliphatic ether  [C-O-C]                    | -0.3474 | -0.3474
MolWt|  * | Molecular Weight Parameter                 |         | -0.1345
Const|  * | Equation Constant                          |         |  0.7475
===========+=========================================+========+=========
   RESULT  |    Biowin1 (Linear Biodeg Probability)    |         |  0.3741
===========+=========================================+========+=========


------+-----+-----------------------------------------+---------+--------
TYPE  | NUM |       Biowin2 FRAGMENT DESCRIPTION       |  COEFF  | VALUE
------+-----+-----------------------------------------+---------+--------
Frag | 1 | Linear C4 terminal chain  [CCC-CH3]        |  1.8437 |  1.8437
Frag | 1 | Aliphatic ether  [C-O-C]                   | -3.4294 | -3.4294
MolWt|  * | Molecular Weight Parameter                 |         | -4.0117
===========+=========================================+========+=========
   RESULT  |  Biowin2 (Non-Linear Biodeg Probability)  |         |  0.0699
===========+=========================================+========+=========
A Probability Greater Than or Equal to 0.5 indicates --> Biodegrades Fast
A Probability Less Than 0.5 indicates --> Does NOT Biodegrade Fast


------+-----+-----------------------------------------+---------+--------
TYPE  | NUM |       Biowin3 FRAGMENT DESCRIPTION       |  COEFF  | VALUE
------+-----+-----------------------------------------+---------+--------
Frag | 1 | Linear C4 terminal chain  [CCC-CH3]        |  0.2983 |  0.2983
Frag | 1 | Aliphatic ether  [C-O-C]                   | -0.0087 | -0.0087
MolWt|  * | Molecular Weight Parameter                 |         | -0.6243
Const|  * | Equation Constant                          |         |  3.1992
===========+=========================================+========+=========
   RESULT  |  Biowin3 (Survey Model - Ultimate Biodeg) |         |  2.8645
===========+=========================================+========+=========


------+-----+-----------------------------------------+---------+--------
TYPE  | NUM |       Biowin4 FRAGMENT DESCRIPTION       |  COEFF  | VALUE
------+-----+-----------------------------------------+---------+--------
Frag | 1 | Linear C4 terminal chain  [CCC-CH3]        |  0.2691 |  0.2691
Frag | 1 | Aliphatic ether  [C-O-C]                   | -0.0097 | -0.0097
MolWt|  * | Molecular Weight Parameter                 |         | -0.4076
Const|  * | Equation Constant                          |         |  3.8477
===========+=========================================+========+=========
   RESULT  |   Biowin4 (Survey Model - Primary Biodeg) |         |  3.6995
===========+=========================================+========+=========


 Result Classification:   5.00 -> hours     4.00 -> days   3.00 -> weeks
   (Primary & Ultimate)    2.00 -> months    1.00 -> longer


------+-----+-----------------------------------------+---------+--------
TYPE  | NUM |       Biowin5 FRAGMENT DESCRIPTION       |  COEFF  | VALUE
------+-----+-----------------------------------------+---------+--------
Frag | 1 | Aliphatic ether  [C-O-C]                   |  0.0015 |  0.0015
Frag | 3 | Methyl  [-CH3]                             |  0.0004 |  0.0012
Frag | 13 | -CH2-  [linear]                           |  0.0494 |  0.6424
Frag | 1 | -CH-   [linear]                            | -0.0507 | -0.0507
Frag | 2 | -CH -  [cyclic]                            |  0.0124 |  0.0249
MolWt|  * | Molecular Weight Parameter                 |         | -0.8405
Const|  * | Equation Constant                          |         |  0.7121
```

Appendix 4-6

```
============+===============================================+=========+=========
   RESULT   |  Biowin5 (MITI Linear Biodeg Probability)  |         |  0.4910
============+===============================================+=========+=========


------+-----+-----------------------------------------------+---------+---------
 TYPE | NUM |        Biowin6 FRAGMENT DESCRIPTION           |  COEFF  |  VALUE
------+-----+-----------------------------------------------+---------+---------
 Frag |  1  |  Aliphatic ether  [C-O-C]                     | -0.1071 | -0.1071
 Frag |  3  |  Methyl  [-CH3]                               |  0.0194 |  0.0583
 Frag | 13  |  -CH2-  [linear]                              |  0.4295 |  5.5834
 Frag |  1  |  -CH-  [linear]                               | -0.0998 | -0.0998
 Frag |  2  |  -CH -  [cyclic]                              | -0.1295 | -0.2589
 MolWt|  *  |  Molecular Weight Parameter                   |         | -8.1558
============+===============================================+=========+=========
   RESULT   |Biowin6 (MITI Non-Linear Biodeg Probability)|         |  0.3883
============+===============================================+=========+=========

 A Probability Greater Than or Equal to 0.5 indicates --> Biodegrades Fast
 A Probability Less Than 0.5 indicates --> Does NOT Biodegrade Fast




AOP Program (v1.91) Results:
===========================
SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
MOL FOR: C19 H38 O1
MOL WT : 282.51
------------------ SUMMARY (AOP v1.91): HYDROXYL RADICALS -----------------
-
Hydrogen Abstraction       =  21.7096 E-12 cm3/molecule-sec
Reaction with N, S and -OH =   0.0000 E-12 cm3/molecule-sec
Addition to Triple Bonds   =   0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =   0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =   0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =   0.0000 E-12 cm3/molecule-sec

   OVERALL OH Rate Constant =  21.7096 E-12 cm3/molecule-sec
   HALF-LIFE =     0.493 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =     5.912 Hrs
------------------ SUMMARY (AOP v1.91): OZONE REACTION --------------------
-

              ******  NO OZONE REACTION ESTIMATION ******
              (ONLY Olefins and Acetylenes are Estimated)

Experimental Database:  NO Structure Matches




PCKOC Program (v1.66) Results:
===========================
```

```
                    Koc (estimated): 3.44e+004


SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
MOL FOR: C19 H38 O1
MOL WT : 282.51
------------------------ PCKOCWIN v1.66 Results ------------------------
-

        First Order Molecular Connectivity Index  ........... :  9.736
        Non-Corrected Log Koc  .............................. :  5.8004
        Fragment Correction(s):
                1   Ether, aliphatic  (-C-O-C-)  .......... : -1.2643
        Corrected Log Koc  .................................. :  4.5361

                      Estimated Koc:  3.437e+004




HYDROWIN Program (v1.67) Results:
==============================
SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
MOL FOR: C19 H38 O1
MOL WT : 282.51
------------------------ HYDROWIN v1.67 Results ------------------------
-

NOTE: Fragment(s) on this compound are NOT available from the fragment
    library. Substitute(s) have been used!!!  Substitute R1, R2, R3,
    or R4 fragments are marked with double astericks "**".


                     O
              R1  / \  R3
EPOXIDE:          >C - C<
              R2        R4
        ** R1: n-Octyl-              ** R3: n-Butyl-
           R2: -H                       R4: -H
 Ka hydrolysis at (epoxy O) atom #  1:  4.271E-001  L/mol-sec

 Total Ka (acid-catalyzed) at 25 deg C :  4.271E-001  L/mol-sec
 Ka Half-Life at pH 7:     187.803  days

   The rate constant estimated for the EPOXIDE DOES NOT
   include the neutral hydrolysis rate constant!!
   For some epoxides, the neutral rate constant is the
   dominant hydrolysis rate at environmental pHs!
   If the neutral rate constant is important, the HYDRO
   estimated rate will under-estimate the actual rate!
```

```
BCF Program (v2.15) Results:
===========================
SMILES : O(C1CCCCCCCCCC)C1CCCCC(C)C
CHEM   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
MOL FOR: C19 H38 O1
MOL WT : 282.51
------------------------------ Bcfwin v2.15 -----------------------------
--
Log Kow  (estimated) :  8.08
Log Kow (experimental):  not available from database
Log Kow used by BCF estimates:  8.08

Equation Used to Make BCF estimate:
   Log BCF = -1.37 log Kow + 14.4 + Correction

      Correction(s):                    Value
       Alkyl chains (8+ -CH2- groups)  -1.500

   Estimated Log BCF =  1.827  (BCF = 67.08)




                   Volatilization From Water
                   =========================

Chemical Name: Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-

Molecular Weight    :  282.51 g/mole
Water Solubility    :  -----
Vapor Pressure      :  -----
Henry's Law Constant:  0.0149 atm-m3/mole  (estimated by Bond SAR Method)

                         RIVER             LAKE
                         ---------         ---------
Water Depth     (meters):  1                1
Wind Velocity   (m/sec):   5                0.5
Current Velocity (m/sec):  1                0.05

      HALF-LIFE (hours) :  1.781            160.4
      HALF-LIFE (days ) :  0.07422          6.682


STP Fugacity Model:  Predicted Fate in a Wastewater Treatment Facility
======================================================================
   (using 10000 hr Bio P,A,S)
PROPERTIES OF: Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
-------------
Molecular weight (g/mol)                                282.51
Aqueous solubility (mg/l)                               0
Vapour pressure (Pa)                                    0
              (atm)                                     0
              (mm Hg)                                   0
Henry 's law constant (Atm-m3/mol)                      0.0149
```

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```
Air-water partition coefficient                          0.609366
Octanol-water partition coefficient (Kow)                1.20226E+008
Log Kow                                                  8.08
Biomass to water partition coefficient                   2.40453E+007
Temperature [deg C]                                      25
Biodeg rate constants (h^-1),half life in biomass (h) and in 2000 mg/L MLSS
(h):
        -Primary tank        0.00      9999.79      10000.00
        -Aeration tank       0.00      9999.79      10000.00
        -Settling tank       0.00      9999.79      10000.00


  STP Overall Chemical Mass Balance:
  -------------------------------
                           g/h            mol/h          percent

Influent                 1.00E+001        3.5E-002        100.00

Primary sludge           5.99E+000        2.1E-002         59.88
Waste sludge             3.33E+000        1.2E-002         33.28
Primary volatilization   2.72E-005        9.6E-008          0.00
Settling volatilization  6.01E-005        2.1E-007          0.00
Aeration off gas         9.17E-003        3.2E-005          0.09

Primary biodegradation   1.75E-002        6.2E-005          0.18
Settling biodegradation  4.25E-003        1.5E-005          0.04
Aeration biodegradation  5.60E-002        2.0E-004          0.56

Final water effluent     5.97E-001        2.1E-003          5.97

Total removal            9.40E+000        3.3E-002         94.03
Total biodegradation     7.77E-002        2.8E-004          0.78


STP Fugacity Model:  Predicted Fate in a Wastewater Treatment Facility
=====================================================================
   (using Biowin/EPA draft method)
PROPERTIES OF: Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
-------------
Molecular weight (g/mol)                                 282.51
Aqueous solubility (mg/l)                                0
Vapour pressure (Pa)                                     0
             (atm)                                       0
             (mm Hg)                                     0
Henry 's law constant (Atm-m3/mol)                       0.0149
Air-water partition coefficient                          0.609366
Octanol-water partition coefficient (Kow)                1.20226E+008
Log Kow                                                  8.08
Biomass to water partition coefficient                   2.40453E+007
Temperature [deg C]                                      25
Biodeg rate constants (h^-1),half life in biomass (h) and in 2000 mg/L MLSS
(h):
        -Primary tank        0.02       30.00        30.00
        -Aeration tank       0.23        3.00         3.00
        -Settling tank       0.23        3.00         3.00
```

```
STP Overall Chemical Mass Balance:
---------------------------------
                              g/h              mol/h           percent

Influent                    1.00E+001          3.5E-002        100.00

Primary sludge              3.78E+000          1.3E-002         37.84
Waste sludge                3.83E-002          1.4E-004          0.38
Primary volatilization      1.72E-005          6.1E-008          0.00
Settling volatilization     6.92E-007          2.4E-009          0.00
Aeration off gas            1.14E-004          4.0E-007          0.00

Primary biodegradation      3.69E+000          1.3E-002         36.91
Settling biodegradation     1.63E-001          5.8E-004          1.63
Aeration biodegradation     2.32E+000          8.2E-003         23.16

Final water effluent        6.87E-003          2.4E-005          0.07

Total removal               9.99E+000          3.5E-002         99.93
Total biodegradation        6.17E+000          2.2E-002         61.70
 (** Total removal recommended maximum is 99 percent)


Level III Fugacity Model (Full-Output):
=======================================
  Chem Name   : Oxirane, 2-decyl-3-(5-methylhexyl)-, cis-
  Molecular Wt: 282.51
  Henry's LC  : 0.0149 atm-m3/mole (Henrywin program)
  Vapor Press : 0.000342 mm Hg  (Mpbpwin program)
  Liquid VP   : 0.00089 mm Hg   (super-cooled)
  Melting Pt  : 67 deg C (Mpbpwin program)
  Log Kow     : 8.08  (Kowwin program)
  Soil Koc    : 4.93e+007  (calc by model)


         Mass Amount    Half-Life    Emissions
          (percent)       (hr)        (kg/hr)
  Air       0.395         11.8          1000
  Water     3.77          360           1000
  Soil      28.1          720           1000
  Sediment  67.8          3.24e+003     0

            Fugacity    Reaction    Advection    Reaction    Advection
             (atm)      (kg/hr)      (kg/hr)     (percent)   (percent)
  Air       1.26e-011    857          146          28.6        4.88
  Water     4.55e-010    269          140          8.96        4.66
  Soil      2.57e-012    1e+003       0            33.4        0
  Sediment  2.8e-010     537          50.2         17.9        1.67

  Persistence Time: 1.24e+003 hr
  Reaction Time:    1.39e+003 hr
  Advection Time:   1.1e+004 hr
  Percent Reacted:  88.8
  Percent Advected: 11.2
```

```
Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
   Air:       11.82
   Water:     360
   Soil:      720
   Sediment: 3240
     Biowin estimate: 2.865  (weeks       )

Advection Times (hr):
   Air:       100
   Water:     1000
   Sediment: 5e+004
```

-

Appendix I
Diflubenzuron
Risk Assessment

Figure I-1. The first power spraying apparatus was used in gypsy moth control operations before 1900.

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

Prepared for:

USDA, Forest Service
Forest Health Protection

GSA Contract No. **GS-10F-0082F**
USDA Forest Service BPA: **WO-01-3187-0150**
Requisition No.: **43-3187-1-0269**
Task No. **5**

Submitted to:
Dave Thomas, COTR
Forest Health Protection Staff
USDA Forest Service
Rosslyn Plaza Building C, Room 7129C
1601 North Kent Street
Arlington, VA 22209

Submitted by:
Patrick R. Durkin
Syracuse Environmental Research Associates, Inc.
5100 Highbridge St., 42C
Fayetteville, New York 13066-0950
Telephone: (315) 637-9560
Fax: (315) 637-0445
E-Mail: SERA_INC@msn.com
Home Page: www.sera-inc.com

July 30, 2004

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NOTE: Tables followed by figures are placed after Section 5 (References).

LIST OF WORKSHEETS

Supplement 1: Diflubenzuron – Worksheets for Human Health and Ecological Risk
Assessments, SERA EXWS 04-43-05-03b1, Version 3.01.

Supplement 2: 4-Chloroaniline as an Environmental Metabolite of Diflubenzuron -Worksheets
for Human Health and Ecological Risk Assessments, SERA EXWS
04-43-05-03b2, Version 3.01.

Located at: http://www.fs.fed.us/foresthealth/pesticide/risk.shtml

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AEL	adverse-effect level
a.i.	active ingredient
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
bw	body weight
CBI	confidential business information
CI	confidence interval
cm	centimeter
CNS	central nervous system
CPU	chlorophenylurea
DAA	days after application
DAT	days after treatment
d.f.	degrees of freedom
EC_x	concentration causing X% inhibition of a process
EC_{25}	concentration causing 25% inhibition of a process
EC_{50}	concentration causing 50% inhibition of a process
EXAMS	Exposure Analysis Modeling System
ExToxNet	Extension Toxicology Network
F	female
FH	Forest Health
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FOIA	Freedom of Information Act
FQPA	Food Quality Protection Act
g	gram
GLEAMS	Groundwater Loading Effects of Agricultural Management Systems
ha	hectare
Hb	hemoglobin
HQ	hazard quotient
IAA	indole-3-acetic acid
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
k_a	absorption coefficient
k_e	elimination coefficient
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
$K_{o/w}$	octanol-water partition coefficient
K_p	skin permeability coefficient
L	liter
lb	pound
LC_{50}	lethal concentration, 50% kill
LD_{50}	lethal dose, 50% kill

ACRONYMS, ABBREVIATIONS, AND SYMBOLS (*continued*)

LOAEL	lowest-observed-adverse-effect level
LOC	level of concern
m	meter
M	male
MetHb	methemoglobinemia
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
mM	millimole
MOS	margin of safety
MRID	Master Record Identification Number
MSDS	material safety data sheet
MW	molecular weight
NCAP	Northwest Coalition for Alternatives to Pesticides
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOS	not otherwise specified
NRC	National Research Council
NTP	National Toxicology Program
OM	organic matter
OPP	Office of Pesticide Programs
OPPTS	Office of Pesticide Planning and Toxic Substances
OSHA	Occupational Safety and Health Administration
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PRZM	Pesticide Root Zone Model
RBC	red blood cells
RED	re-registration eligibility decision
RfD	reference dose
SERA	Syracuse Environmental Research Associates
SRC	Syracuse Research Corporation
UF	uncertainty factor
U.S.	United States
USDA	U.S. Department of Agriculture
U.S. EPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WCR	water contamination rate
WHO	World Health Organization
WP	wettable powder
μ	micron or micro-
4CA	4-chloroaniline

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

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

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

CONVERSION OF SCIENTIFIC NOTATION

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

EXECUTIVE SUMMARY

OVERVIEW
While the data base supporting the risk assessment of diflubenzuron is large and somewhat complex, the risk characterization is relatively simple. Diflubenzuron is an effective insecticide. Consequently, application rates used to control the gypsy moth are likely to have effects on some nontarget terrestrial insects. Species at greatest risk include grasshoppers, various macrolepidoptera (including the gypsy moth), other herbivorous insects, and some beneficial predators of the gypsy moth. Some aquatic invertebrates may also be at risk; however, the risks appear to be less severe than risks to terrestrial insects. The risk characterization for aquatic invertebrates is highly dependant on site-specific conditions. In areas subject to minimal water contamination, the effects of diflubenzuron are expected to be marginally adverse or nonexistent. If diflubenzuron is applied when drift or direct deposition in water is not controlled well or in areas where soil losses from runoff and sediment to water are likely to occur, certain aquatic invertebrates are at risk of acute adverse effects, and exposure could cause longer-term effects on more sensitive species. Direct effects of diflubenzuron on humans and other groups of organisms—wildlife mammals, birds, amphibians, fish, terrestrial and aquatic plants, microorganisms, and non-arthropod invertebrates—do not appear to be plausible. Nontarget species that consume the gypsy moth or other invertebrates adversely affected by diflubenzuron may be at risk of secondary effects of exposure (for example, a change in the availability of prey). There is no indication that 4-chloroaniline formed from the degradation of diflubenzuron will have an adverse effect on any species.

PROGRAM DESCRIPTION
Diflubenzuron is an insecticide that inhibits chitin deposition in arthropods and is effective either as a stomach or contact insecticide. Two formulations of diflubenzuron are labeled for control of the gypsy moth: Dimilin 4L and Dimilin 25W. Other formulations of diflubenzuron are available but these are registered for agricultural uses which account for about 94% of the total amount of diflubenzuron applied each year. Both ground and aerial applications of Dimilin 4L and Dimilin 25W are permitted. The current risk assessment concerns the range of labeled application rates—i.e., 0.0078-0.0624 lbs a.i./acre. Virtually all use of diflubenzuron in USDA programs occurs in suppression programs (about 99% of the treated acres) with only about 1% of the use in slow the spread programs. The use of diflubenzuron in eradication programs is less than 0.001% of the total use.

HUMAN HEALTH RISK ASSESSMENT
Hazard Identification – There is no information regarding effects in humans exposed to diflubenzuron; however, the toxicity of this compound is well characterized in experimental mammals. In mammals, the most sensitive effect involves damage to hemoglobin, a component of blood involved in the transport of oxygen. Diflubenzuron causes the formation of methemoglobin, a form of hemoglobin that is not able to transport oxygen. Methemoglobinemia, an excessive formation of methemoglobin, is the primary toxic effect of diflubenzuron regardless of the route or duration of exposure in every species of animal tested. Diflubenzuron causes

other effects on the blood; however, methemoglobinemia is the most sensitive effect—that is, the effect that occurs at the lowest dose. While effects on the blood are well documented, there is little indication that diflubenzuron causes other specific forms of toxicity. Diflubenzuron does not appear to be neurotoxic or immunotoxic, does not appear to affect endocrine function in laboratory mammals, and is not a carcinogen. In addition, diflubenzuron does not appear to cause birth defects or reproductive effects. Diflubenzuron is relatively nontoxic by oral administration, with reported single-dose LD_{50} values ranging from greater than 4640 to greater than10,000 mg/kg. There are numerous studies regarding the subchronic and chronic toxicity of diflubenzuron in laboratory animals, and these studies indicate that methemoglobinemia is the most consistent and sensitive sign of toxicity. Diflubenzuron can be absorbed from the skin in sufficient amounts to cause hematological effects—that is, methemoglobinemia and sulfhemoglobinemia. Nonetheless, the dermal exposure concentrations that are necessary to cause these hematological effects are higher than the oral exposure doses that are necessary to cause the same effects.

Exposure Assessment – Exposure assessments are conducted for both diflubenzuron and 4-chloroaniline. For diflubenzuron, a standard set of exposure scenarios are presented for both workers and members of the general public. Concern for 4-chloroaniline arises because it is an environmental metabolite of diflubenzuron and is classified as a carcinogen. 4-Chloroaniline is not a concern in worker exposure assessments because 4-chloroaniline will not be present at the time that diflubenzuron is applied. Also, 4-chloroaniline is not a concern in some acute exposure scenarios for the general public such as direct spray during the application of diflubenzuron. Consequently, only a subset of the standard exposure scenarios—those associated with exposure to vegetation or water contaminated with diflubenzuron—are presented for 4-chloroaniline. These scenarios, however, include all standard chronic exposure scenarios, which are of greatest concern because of the potential carcinogenicity of 4-chloroaniline.

All exposure assessments are conducted at the maximum single application rate for diflubenzuron of 0.0625 lb/acre (equivalent to 70 g/ha). This is also the maximum application rate for a single season. Assuming that diflubenzuron is applied in a single application at the maximum rate leads to the highest estimates of peak as well as longer-term exposures. The consequences of using lower application rates are discussed in the risk characterization.

For workers applying diflubenzuron, three types of application methods are considered: directed ground spray, broadcast ground spray, and aerial spray. Central estimates of exposure for workers are approximately 0.0009 mg/kg/day for aerial workers, 0.0008 mg/kg/day for backpack workers, and about 0.001 mg/kg/day for broadcast ground spray workers. Upper ranges of exposures are approximately 0.009 mg/kg/day for broadcast ground spray workers and 0.005 mg/kg/day for backpack and aerial workers. All of the accidental exposure scenarios for workers involve dermal exposures, and most of these accidental exposures lead to dose estimates that are either in the range of or substantially below the general exposure estimates for workers. The one exception involves wearing contaminated gloves for 1hour. The upper range of exposure for this scenario is about 0.4 mg/kg/day.

For the general public, estimates of acute exposure range from approximately 0.0000005 mg/kg, which is the lower range estimate for the consumption by a child of water from a stream contaminated by diflubenzuron, to 1.5 mg/kg, which represents the upper range for consumption of contaminated fish by subsistence populations—individuals who consume free-caught fish as a major proportion of their diet. Relatively high dose estimates are also associated with the consumption of contaminated water after an accidental spill (about 0.13 mg/kg at the upper range of exposure) and for the consumption of fish by members of the general public (0.3 mg/kg). Other acute exposures are lower by about an order of magnitude or more. For chronic or longer-term exposures, the modeled exposures are much lower than for acute exposures, ranging from approximately 0.00000002 mg/kg/day (2 in 10 millionths of a mg/kg/day), which is the lower range estimate for the consumption of contaminated water, to approximately 0.002 mg/kg/day, which is the upper range for consumption of contaminated fruit.

Estimates of exposure to 4-chloroaniline from contaminated vegetation are likely to be about 0.02 times less than corresponding estimates of exposure to diflubenzuron. The lower estimate of exposure to 4-chloroaniline is due to its expected rapid dissipation from diflubenzuron deposited on vegetation. In water, however, estimated concentrations of 4-chloroaniline are likely to be equal to or greater than anticipated water concentrations of diflubenzuron under certain circumstances. Finally, peak exposures to 4-chloroaniline differ from peak exposures to diflubenzuron in the environment, usually occurring at different times (later after the application of diflubenzuron) and under different conditions of precipitation. These differences are due to the relatively slow rate in the formation of 4-chloroaniline from diflubenzuron in soil.

Dose-Response Assessment – The dose-response assessment considers both diflubenzuron itself as well as 4-chloroaniline as an environmental metabolite of diflubenzuron. For systemic toxicity, the dose-response assessment involves the adoption or derivation of acute and chronic RfDs, doses that are considered to produce no adverse effects, even in sensitive individuals. RfDs are presented for both diflubenzuron and 4-chloroaniline. Cancer risk is considered quantitatively for 4-chloroaniline and is expressed as a dose associated with a risk of 1 in 1million. Following standard practices for USDA risk assessments, risk assessment values available from U.S. EPA are adopted directly unless there is a compelling basis for doing otherwise. When risk values are not available from U.S. EPA, the methods used by U.S. EPA are employed to derive surrogate values.

U.S. EPA derived a chronic RfD for diflubenzuron of 0.02 mg/kg/day. This chronic RfD is well documented and is used directly for all longer-term exposures to diflubenzuron. This value is based on a NOAEL in dogs and an uncertainty factor of 100. Because of the low acute toxicity of diflubenzuron, the U.S. EPA did not derive an acute RfD but identified an acute NOAEL of 10,000 mg/kg. While this NOAEL could be used to derive a surrogate acute RfD of 100 mg/kg, a more conservative approach is taken and a surrogate acute RfD of 11 mg/kg is derived based on a NOAEL of 1118 mg/kg from a study using a petroleum-based formulation of diflubenzuron. Since diflubenzuron is classified as a non-carcinogen by both U.S. EPA and WHO, there is no reason to conduct a quantitative cancer risk assessment for exposure to diflubenzuron.

The U.S. EPA derived a chronic RfD for 4-chloroaniline of 0.004 mg/kg/day, and this value is used in the current risk assessment to characterize risks from 4-chloroaniline for longer-term exposures. This RfD is based on a chronic oral LOAEL of 12.5 mg/kg/day using an uncertainty factor of 3000—three factors of 10 each for intraspecies extrapolation, sensitive subgroups, and the use of a LOAEL with an additional factor of 3 due to the lack of data reproductive toxicity data. As with diflubenzuron, the U.S. EPA did not derived an acute RfD for 4-chloroaniline. For this risk assessment a conservative approach is taken in which a surrogate acute RfD of 0.03 mg/kg is based on a subchronic (90-day) NOAEL of 8 mg/kg/day. Consistent with the approach taken by U.S. EPA for the chronic RfD, an uncertainty factor of 300 is used—a factor of 10 for interspecies extrapolation, 10 for intraspecies extrapolation, and 3 for the lack of data on reproductive toxicity. For cancer risk, the U.S. EPA proposes a human cancer potency factor for 4-chloroaniline of 0.0638 $(mg/kg/day)^{-1}$. This potency factor is used to calculate a dose of 1.6×10^{-5} mg/kg/day that would be associated with a cancer risk of 1 in 1million.

Risk Characterization – The risk characterization for potential human health effects associated with the use of diflubenzuron in USDA programs to control the gypsy moth is relatively unambiguous: none of the hazard quotients reach a level of concern at the highest application rate that could be used in USDA programs. In that many of the exposure assessments involve very conservative assumptions—that is, assumptions that tend to overestimate exposure—and because the dose-response assessment is based on similarly protective assumptions, there is no basis for asserting that this use of diflubenzuron poses a hazard to human health.

Notwithstanding the above assertion, it is worth noting that the greatest relative risk concerns the contamination of water with 4-chloroaniline rather than exposure to diflubenzuron itself. The highest hazard quotient for diflubenzuron is 0.1, a factor of 10 below a level of concern. Since this hazard quotient is based on toxicity, an endpoint that is considered to have a population threshold, the assertion can be made that risk associated with exposure to diflubenzuron is essentially zero.

This is not the case with 4-chloroaniline, which is classified as a probable human carcinogen and is an environmental metabolite of diflubenzuron. For 4-chloroaniline, the highest hazard quotient is 0.4, below the level of concern by a factor of only 2.5. The scenario of greatest concern involves cancer risk from drinking contaminated water. This risk would be most plausible in areas with sandy soil and annual rainfall rates ranging from about 50 to 250 inches. The central estimate of the hazard quotient for the consumption of water contaminated with 4-chloroaniline and based on a cancer risk of 1 in 1million is 0.09, which is 10 times lower than the level of concern.

ECOLOGICAL RISK ASSESSMENT
Hazard Identification – The toxicity of diflubenzuron is well characterized in most groups of animals, including mammals, birds, terrestrial invertebrates, fish, and aquatic invertebrates. In general, diflubenzuron is much more toxic to some invertebrates, specifically arthropods, than vertebrates or other groups of invertebrates. This differential toxicity appears to involve

fundamentally different mechanisms of action. Toxicity to sensitive invertebrate species is based on the inhibition of chitin synthesis. In the more tolerant vertebrate species, the mechanism of action appears to be a specific effect on the blood that inhibits oxygen transport.

The species most sensitive to diflubenzuron are arthropods, a large group of invertebrates, including insects, crustaceans, spiders, mites, and centipedes. Most of these organisms use chitin, a polymer (repeating series of connected chemical subunits) of a glucose-based molecule, as a major component of their exoskeleton—that is, outer body shell.   Diflubenzuron is an effective insecticide because it inhibits the the formation of chitin. This effect disrupts the normal growth and development of insects and other arthropods. Both terrestrial and aquatic arthropods are affected but some substantial differences in sensitivity are apparent. In terrestrial organisms, the most sensitive species include lepidopteran and beetle larvae, grasshoppers and other herbivorous insects. More tolerant species include bees, flies, parasitic wasps, adult beetles, and sucking insects. In aquatic organisms, small crustaceans that consume algae and serve as a food source for fish (e.g., *Daphnia* species) appear to be the most sensitive to diflubenzuron, while larger insect species such as backswimmers and scavenger beetles are much less sensitive. A wide range of other aquatic invertebrates, other crustaceans ,and small to medium sized aquatic insect larvae, appear to have intermediate sensitivities. Not all invertebrates use chitin and these invertebrates are much less sensitive to diflubenzuron than the arthropods. For terrestrial invertebrates, relatively tolerant species include earthworms and snails. For aquatic species, tolerant species include ostracods and non-arthropods such as rotifers, bivalves (clams), aquatic worms, and snails.

The most sensitive effect in vertebrate species concerns damage to blood cells involved in the transport of oxygen. This effect was demonstrated in laboratory mammals used in toxicity studies (for example, rats and mice) as well as in domestic animals and livestock. Although the effect was not studied in wildlife mammals, birds, or fish, it is reasonable to assume that hemoglobin in all vertebrate species could be affected by exposure to diflubenzuron. Acute exposures to diflubenzuron are relatively non-toxic to mammals and birds. The U.S. EPA places diflubenzuron in low toxicity categories (III or IV) for mammals and considers diflubenzuron to be virtually non-toxic to birds in acute exposures and only slightly toxic to birds in subchronic exposures. This assessment is supported by a numerous field studies in which no direct toxic effects in mammals or birds is reported. Effects, if any, on terrestrial vertebrates from the application of diflubenzuron are likely to be secondary to changes in food availability—that is, reduced numbers of insects—or changes in habitat— for example, the loss of protective vegetation, relative to areas not treated with diflubenzuron. Aquatic vertebrates also appear to be relatively tolerant to diflubenzuron ,and this compound is classified by U.S. EPA as practically non-toxic to fish. This classification appears to be appropriate and is supported by several longer-term toxicity studies and field studies. Changes in fish populations are reported in some studies; however, the changes appear to be secondary to changes in food supply. Although the data on amphibians is much more limited than the data onfish, a similar pattern is apparent—that is, although there are no direct toxic effects from exposure, changes in food consumption patterns appear secondary to direct effects on invertebrate species.

Data on plants and microorganisms are more limited than the data on invertebrates or vertebrates. Nonetheless, there does not appear to be any basis for asserting that diflubenzuron will have a substantial effect on these organisms.

Exposure Assessment – As in the human health risk assessment, exposures are estimated for both diflubenzuron and 4-chloroaniline. A full set of exposure assessments are developed for diflubenzuron but only a subset of exposure assessments are developed for 4-chloroaniline. This approach is taken, again as in the human health risk assessment, because 4-chloroaniline is assessed as an environmental metabolite of diflubenzuron. Thus, immediately after application, the amount of 4-chloroaniline as an environmental metabolite will be negligible. Consequently, the direct spray scenarios as well as the consumption of insects and the consumption of small mammals after a direct spray are not included for 4-chloroaniline. Also as in the human health risk assessment, all standard chronic exposure scenarios are included for 4-chloroaniline.

Terrestrial animals might be exposed to any applied pesticide from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation. For diflubenzuron, the highest acute exposures for small terrestrial vertebrates will occur after a direct spray and could reach up to about 10 mg/kg at an application rate of 70 g/ha. Exposures anticipated from the consumption of contaminated vegetation by terrestrial animals range from central estimates of about 0.08 mg/kg for a small mammal to 2 mg/kg for a large bird with upper ranges of about 0.2 mg/kg for a small mammal and 5 mg/kg for a large bird. The consumption of contaminated water leads to much lower levels of exposure. A similar pattern is seen for chronic exposures. Estimated longer-term daily doses for the a small mammal from the consumption of contaminated vegetation at the application site range from approximately 0.001 to 0.005 mg/kg. Large birds feeding on contaminated vegetation at the application site could be exposed to much higher concentrations, ranging from about 0.08 to 0.7 mg/kg/day. The upper ranges of exposure from contaminated vegetation far exceed doses anticipated from the consumption of contaminated water, which range from about 0.0000001 to 0.00001 mg/kg/day for a small mammal.

Exposures of terrestrial organisms to 4-chloroaniline tend to be much lower than those for diflubenzuron. The highest acute exposure is about 0.2 mg/kg, the approximate dose for the consumption of contaminated water by a small mammal and the consumption of contaminated fish by a predatory bird. The highest longer term exposure is 0.0002 mg/kg/day, the dose associated with the consumption of contaminated vegetation by a large bird.

Exposures to aquatic organisms are based on essentially the same information used to assess the exposure to terrestrial species from contaminated water. At the maximum application rate of 70 g/ha, the upper range of the expected peak concentration of diflubenzuron in surface water is taken as 16 µg/L. The lower range of the concentration in ambient water is estimated at 0.01 µg/L. The central estimate of concentration of diflubenzuron in surface water is taken as 0.4 µg/L.

Dose-Response Assessment – Diflubenzuron is relatively non-toxic to mammals and birds. For mammals, the toxicity values used in the ecological risk assessment are identical to those used in the human health risk assessments: an acute NOAEL of 1118 mg/kg and a chronic NOAEL of 2 mg/kg/day.   A similar approach is taken for 4-chloroaniline for which an acute NOAEL is 8 mg/kg is used based on a subchronic study and a chronic NOAEL is estimated at 1.25 mg/kg/day based on the chronic LOAEL of 12.5 mg/kg/day. For birds, the acute NOAEL for diflubenzuron is taken as 2500 mg/kg from an acute gavage study and the longer-term NOAEL is taken as 110 mg/kg/day from a reproduction study. No data are available regarding the toxicity of 4-chloroaniline in birds and the available toxicity values for mammals are used as a surrogate.

For terrestrial invertebrates two general types of data could be used to assess dose-response relationships: laboratory toxicity studies and field studies. Field studies are used in the current risk assessment because the standard toxicity studies are extremely diverse and many are not directly applicable to a risk assessment. Despite the difficulty and uncertainty in interpreting some of the field studies, the relatively large number of field studies on diflubenzuron appear to present a reasonably coherent pattern that is at least qualitatively consistent with the available toxicity data and probably a more realistic basis on which to assess risk to nontarget species. The most sensitive species appear to be grasshoppers which may be adversely affected at an application rate of 22 g/ha. Somewhat high application rates—in the range of 30 to 35 g/ha—will adversely effect macrolepidoptera and some beneficial parasitic wasps. At the maximum application rate considered in this risk assessment— 70 g/ha—some herbivorous insects are likely to be affected. No adverse effects in several other groups of insects are expected at this or much higher application rates. Honeybees are among the most tolerant species and are not likely to be adversely affected at application rates of up to 400 g/ha.

Invertebrates that do not synthesize chitin are also relatively tolerant to diflubenzuron.   The NOEC for a species of earthworm (*Eisenia fetida*) is 780 mg/kg soil and is used to represent tolerant species of soil invertebrates. Very little information is available on the toxicity of 4-chloroaniline to terrestrial invertebrates.   As with diflubenzuron, the earthworm appears to be relatively tolerant to 4-chloroaniline with a reported LC_{50} value of 540 mg/kg dry soil. The toxicity of both diflubenzuron and 4-chloroaniline to soil microorganisms is also relatively low.

Toxicity values for aquatic species follow a pattern similar to that for terrestrial species: arthropods appear to be much more sensitive than fish or non-arthropod invertebrates.   For diflubenzuron, LC_{50} values of 25-500 mg/L are used to characterize risks for sensitive and tolerant species of fish, respectively. 4-Chloroaniline appears to be more toxic to fish and an LC_{50} of 2.4 mg/L is used to characterize risks of peak exposures, while an LC_{50} of 0.2 mg/L is used to characterize risks of longer-term exposures.

There is substantial variability in the response of different groups of aquatic invertebrates to diflubenzuron. Very small arthropods appear to be among the most sensitive species—with acute NOEC values ranging from 0.3 to about 1 ppb (µg/L) and chronic NOEC values ranging from 0.04 to 0.25 ppb. Based on acute NOEC values, larger arthropods, including crabs and

larger insects, appear to be more tolerant, with acute NOEC values ranging from 2 to 2000 ppb. For chronic effects, the differences between small and larger arthropods are less remarkable with stoneflies and mayflies (relatively large insects) having an NOEC value of 0.1 ppb, intermediate between *Daphnia* (0.04 ppb) and *Ceriodaphnia* (0.25 ppb). Molluscs (invertebrates including clams and snails) and worms (oligochaetes) appear to be much less sensitive to diflubenzuron.

The data on the toxicity of 4-chloroaniline to aquatic invertebrates is sparse. An acute NOEC of 0.013 mg/L is used to characterize acute risks associated with peak exposures in aquatic invertebrates, and an NOEC of 0.01 mg/L from a reproduction study is used to characterize longer-term risks to aquatic invertebrates.

Risk Characterization – While the data base supporting the risk assessment of diflubenzuron is large and somewhat complex, the risk characterization is relatively simple. Diflubenzuron is an effective insecticide. Consequently, application rates used to control the gypsy moth are likely to have effects on some nontarget terrestrial insects. Species at greatest risk include grasshoppers, various macrolepidoptera (including the gypsy moth), other herbivorous insects, and some beneficial predators to the gypsy moth. These species are at risk because of the mode of action of diflubenzuron (i.e., inhibition of chitin) and the behavior of the sensitive insects (the consumption of contaminated vegetation or predation on the gypsy moth). Some aquatic invertebrates may also be at risk but the risks appear to be less than risks to terrestrial insects. The risk characterization for aquatic invertebrates is highly dependant on site-specific conditions. If diflubenzuron is applied when drift or direct deposition in water is not controlled well or in areas where soil losses from runoff and sediment to water are likely to occur, certain aquatic invertebrates are at risk of acute adverse effects, and exposure could cause longer-term effects on more sensitive species.

Direct effects of diflubenzuron on other groups of organisms—that is, mammals, birds, amphibians, fish, terrestrial and aquatic plants, microorganisms, and non-arthropod invertebrates—do not appear to be plausible. Nontarget species that consume the gypsy moth or other invertebrates adversely affected by diflubenzuron may be at risk of secondary effects of exposure (for example, a change in the availability of prey). There is no indication that 4-chloroaniline formed from the degradation of diflubenzuron will have an adverse effect on any species

There is no indication that 4-chloroaniline formed from the degradation of diflubenzuron will have an adverse effects on any species.

1. INTRODUCTION

This document provides updated risk assessments for human health effects and ecological effects to support an assessment of the environmental consequences of using diflubenzuron for the control or eradication of the gypsy moth (*Lymantria dispar*) in USDA/Forest Service and USDA/APHIS programs.   This risk assessment is an update to the human health and ecological risk assessments prepared for the 1995 Final Environmental Impact Statement (FEIS) for the Cooperative Gypsy Moth Management Program (USDA 1995).

In the preparation of this risk assessment, literature searches on diflubenzuron were conducted using PubMed, TOXLINE, AGRICOLA, as well as the U.S. EPA CBI files. There is a very large body of literature on the environmental fate and toxicology of diflubenzuron. In addition to the previous risk assessments (USDA 1995), the toxicology, environmental fate, and other aspects associated with the use of diflubenzuron are the subject of relatively comprehensive reviews of human health and ecological effects by the World Health Organization (WHO 1996; WHO 2001). Several other reviews of various topics involving diflubenzuron have been published in the open literature (e.g. Cunningham 1986; Eisler 1992; Fisher and Hall 1992; Wilson 1997) and in materials submitted to U.S. EPA (Cardona 1999; Hobson 2001; Lengen, 1999; Wilcox and Coffey 1978).

In addition, a large number of studies have been submitted to the U.S. EPA/OPP in support of the registration of diflubenzuron and most of these studies have been reviewed by U.S. EPA (U.S. EPA/OPP 1997a, 1997b, 2000) and the derivation of food tolerances (EPA/OPP 1999, 2002a, 2003). The U.S. EPA (1997a) re-registration eligibility decision (RED) document and other reviews by U.S. EPA include summaries of the product chemistry, mammalian toxicology, and ecotoxicology studies that were submitted by industry to the U.S. EPA. Full text copies of the studies most relevant to this risk assessment (n=118) were kindly provided by the U.S. EPA Office of Pesticide Programs. The CBI studies were reviewed, and synopses of the information that can be disclosed from these studies are included in this document.

While this document discusses the studies required to support the risk assessments, it makes no attempt to re-summarize all of the information cited in the existing reviews. This is a general approach in all Forest Service risk assessments. For diflubenzuron in particular, an attempt to re-summarize all of the available information would tend to obscure rather than clarify the key studies that should and do impact the risk assessment.

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

For the most part, the risk assessment methods used in this document are similar to those used in risk assessments previously conducted for the Forest Service as well as risk assessments conducted by other government agencies. Details regarding the specific methods used to prepare the human health risk assessment are provided in SERA (2001). This document has four chapters, including the introduction, program description, risk assessment for human health effects, and risk assessment for ecological effects or effects on wildlife species. Each of the two risk assessment chapters has four major sections, including an identification of the hazards associated with diflubenzuron and its commercial formulations, an assessment of potential exposure to the product, an assessment of the dose-response relationships, and a characterization of the risks associated with plausible levels of exposure. These are the basic steps recommended by the National Research Council of the National Academy of Sciences (NRC 1983) for conducting and organizing risk assessments.

Risk assessments are usually expressed with numbers; however, the numbers are far from exact. *Variability* and *uncertainty* may be dominant factors in any risk assessment, and these factors should be expressed. Within the context of a risk assessment, the terms *variability* and *uncertainty* signify different conditions.

Variability reflects the knowledge of how things may change. Variability may take several forms. For this risk assessment, three types of variability are distinguished: *statistical*, *situational*, and *arbitrary*. *Statistical variability* reflects, at least, apparently random patterns in data. For example, various types of estimates used in this risk assessment involve relationships of certain physical properties to certain biological properties. In such cases, best or maximum likelihood estimates can be calculated as well as upper and lower confidence intervals that reflect the statistical variability in the relationships. *Situational variability* describes variations depending on known circumstances. For example, the application rate or the applied concentration of a herbicide will vary according to local conditions and goals. As discussed in the following section, the limits on this variability are known and there is some information to indicate what the variations are. In other words, *situational variability* is not random. *Arbitrary variability*, as the name implies, represents an attempt to describe changes that cannot be characterized statistically or by a given set of conditions that cannot be well defined. This type of variability dominates some spill scenarios involving either a spill of a chemical on to the surface of the skin or a spill of a chemical into water. In either case, exposure depends on the amount of chemical spilled and the area of skin or volume of water that is contaminated.

Variability reflects a knowledge or at least an explicit assumption about how things may change, while *uncertainty* reflects a lack of knowledge. For example, the focus of the human health dose-response assessment is an estimation of an 'acceptable' or 'no adverse effect' dose that will not be associated with adverse human health effects. For diflubenzuron and for most other chemicals, however, this estimation regarding human health must be based on data from experimental animal studies, which cover only a limited number of effects. Generally, judgment is the basis for the methods used to make the assessment. Although the judgments may reflect a consensus (i.e., be used by many groups in a reasonably consistent manner), the resulting

estimations of risk cannot be proven analytically. In other words, the estimates regarding risk involve uncertainty. The primary functional distinction between variability and uncertainty is that variability is expressed quantitatively, while uncertainty is generally expressed qualitatively.

In considering different forms of variability, almost no risk estimate presented in this document is given as a single number. Usually, risk is expressed as a central estimate and a range, which is sometimes very large. Because of the need to encompass many different types of exposure as well as the need to express the uncertainties in the assessment, this risk assessment involves numerous calculations. Some of the calculations are relatively simple are included in the body of the document. Some sets of the calculations, however, are cumbersome. For those calculations, worksheets are included with this risk assessment. The worksheets provide the detail for the estimates cited in the body of the document. Documentation for these worksheets is provided in a separate document (SERA 2003).   A set of worksheets is provided for diflubenzuron (Supplement 1) as well as 4-chloroaniline (Supplement 2). As discussed in this risk assessment, 4-chloroaniline is a metabolite of diflubenzuron that is quantitatively considered in this risk assessment. Both sets of worksheets are provided with the hard-text copy of this risk assessment as well as with the electronic version of the risk assessment.

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

2. PROGRAM DESCRIPTION

2.1. Overview

Diflubenzuron is an insecticide that inhibits chitin deposition in arthropods and is effective either as a stomach or contact insecticide. Two formulations of diflubenzuron are labeled for control of the gypsy moth: Dimilin 4L and Dimilin 25W. Other formulations of diflubenzuron are available but these are registered for agricultural uses, which account for about 94% of the total amount of diflubenzuron applied each year. Both ground and aerial applications of Dimilin 4L and Dimilin 25W are permitted. For the current risk assessment, the range of labeled application rates – i.e., 0.0078 lb a.i./acre to 0.0624 lbs a.i./acre – are considered. Virtually all use of diflubenzuron in USDA programs occurs in suppression programs (about 99% of treated acres) with only about 1% of the use in slow the spread programs. The use of diflubenzuron in eradication programs is less than 0.001% of the total use.

2.2. Chemical Description and Commercial Formulations

Diflubenzuron is the common name for [1-(4-chlorophenyl) 3-(2,6-difluorobenzoyl)urea]:

Structurally, diflubenzuron consists of *p*-chloroanaline (the moiety on the left) linked to a 2,6-difluorobenzoic acid (the moiety on the right) by a ureido (carbon-nitrogen) bridge. Other synonyms for diflubenzuron as well as selected chemical and physical properties of diflubenzuron are summarized in Table 2-1. Additional information on the environmental fate and transport of diflubenzuron is summarized in the exposure assessments for the human health risk assessment (Section 3.2) and ecological risk assessment (Section 4.2).

Diflubenzuron is an insecticide that inhibits chitin deposition in arthropods and is effective either as a stomach or contact insecticide (Mabury and Crosby 1996). Chitin is a polymer (repeating series of connected chemical subunits) of a glucose-based molecule and comprises a substantial proportion of the exoskeleton (outer-shell) of arthropods. Consequently, the inhibition of chitin synthesis disrupts the growth and development (Baishya and Hazarika 1996; DeCleraq et al. 1995a,b; Griffith et al. 1996; Post and others 1974; Wright et al. 1996). Thus, diflubenzuron is not specific to the gypsy moth (Griffith et al. 1996; Horst and Walker 1995; Kadam et al. 1995) and is used to control a variety of pests on a variety of vegetation (Booth Riedl 1996; Boyle et al. 1996; McCasland et al. 1998). Because diflubenzuron can impact a number of invertebrate species, particularly aquatic species (e.g., Liber et al. 1996; O'Halloran et al. 1996), this compound is a restricted use pesticide that may only be applied by licenced applicators (C&P Press 2004).

Various formulations of diflubenzuron are labeled for forestry applications as well as other applications. All formulations of diflubenzuron are currently registered to Uniroyal Chemical (Table 2-2). Two formulations of diflubenzuron are labeled for control of the gypsy moth: Dimilin 4L and Dimilin 25W. As indicated in Table 2-2, an additional formulation, Micromite 25W, had been registered for gypsy moth but this formulation has been discontinued and the registration for this product has been canceled (U.S. EPA/OPP 2002b). Micromite 25WS and Micromite 25WGS are still available but these formulations are not used in USDA programs for the control of the gypsy moth.

Information on the impurities in and composition of these and other formulations of diflubenzuron have been submitted to U.S. EPA/OPP and this information (i.e., Drozdick 1998a,b,c,d,e; Van Kampen and Thus 1996; Vanstone 1998a,b,c; White 1998) has been reviewed as part of the current risk assessment. Specific information on inerts and contaminants in the diflubenzuron formulations is classified as CBI (confidential business Information) under Section 7(d) and Section (10) of FIFRA. This information cannot be specifically disclosed in this risk assessment. WHO (1996) has reported in the open literature that at least some processes in the synthesis diflubenzuron involve the reaction of 2,6-difluoro-benzamide with 4-chlorophenylisocyanate. Some inerts, however, must be disclosed on the material safety data sheet. Dimilin 4L contains petroleum oil [CAS No. 64742-46-7] and Dimilin 25W contains kaolin clay (C&P Press 2004). WHO (1996) indicated that kaolin is the only inert in some formulations of diflubenzuron. The potential risks associated with these inerts in the diflubenzuron formulations are discussed in Section 3.1.14.

2.3. Application Methods and Rates

Both ground and aerial applications of Dimilin 4L and Dimilin 25W are permitted (C&P Press 2004) and both methods are used in USDA programs. The most common methods for ground applications of diflubenzuron are hydraulic sprayers, mist blowers, or air blast sprayers (broadcast foliar). The spray equipment is typically mounted on tractors or trucks used to apply the insecticide on either side of the roadway. Usually, about 8 acres are treated in a 45-minute period (approximately 11 acres/hour). Special truck-mounted spray systems may be used to treat up to 12 acres in a 35-minute period with approximately 300 gallons of insecticide mixture (approximately 21 acres/hour and 510 gallons/hour) (USDA/FS89b, p 2-9 to 2-10).

In some instances, directed foliar applications may be used. In selective foliar applications, backpack applicators are used and the insecticide is applied to target vegetation. Application crews may treat up to shoulder high brush, which means that chemical contact with the arms, hands, or face is plausible. To reduce the likelihood of significant exposure, application crews are directed not to walk through treated vegetation. Usually, a worker treats approximately 0.5 acres/hour with a plausible range of 0.25-1.0 acre/hour.

In aerial applications, diflubenzuron formulations are applied under pressure through specially designed spray nozzles and booms. The nozzles are designed to minimize turbulence and maintain a large droplet size, both of which contribute to a reduction in spray drift. In aerial applications, approximately 40 to 100 acres may be treated per hour (USDA/FS89b, p 2-11). For Dimilin 25W, recommended droplet sizes are in the range of 150 to 200 microns (C&P Press 2004).

As indicated in Table 2-2, the application rate for Dimilin 4L ranges from 0.5 fluids ounces to 2 fluid ounces per acre. This corresponds to about 0.0039 to 0.0156 gallons [128 ounces per gallon] of Dimilin 4L per acre, which in turn corresponds to about 0.0156 to 0.0624 lbs diflubenzuron per acre [4 lbs diflubenzuron per gallon $\times$ 0.0039 to 0.0156] and 17 to 70 grams/ha. While multiple applications are permitted, the maximum single application rate is equal to the maximum annual application rate.

For Dimilin 25W, the range of labeled application rates is 0.5 ounces (avoirdupois) to 2 ounces per acre or 0.03125 to 0.125 pounds of Dimilin 25W per acre [i.e., 16 avoirdupois ounces per pound]. Since Dimilin 25W consists of 25% diflubenzuron, this range of application rates is equivalent to about 0.0078 to 0.03125 lb diflubenzuron per acre and 9 to 35 grams/ha. These rates for Dimilin 25W are about a factor of two below the corresponding rates for Dimilin 4L. The maximum application rate for Dimilin 25W in a single application is equivalent to the maximum annual application rate – i.e., multiple applications are allowed each year but the total amount applied in a single year cannot exceed 0.03125 lb a.i./acre [35 g/ha].

For the current risk assessment, the range of labeled application rates – i.e., 0.0078 lb a.i./acre to 0.0624 lbs a.i./acre – are considered. As calculated above, these rates are equivalent to 9 g/ha to 70 g/ha. All exposure assessments will be conducted at the maximum application rate. The consequences of using lesser rates are considered further in the risk characterization for human health (Section 3.4) and ecological effects (Section 4.4). These application rates are essentially the same as those used in the previous risk assessment (USDA 1995).

Recommended high volume ground sprays of Dimilin 4L and Dimilin 25W typically involve 100 to 400 gallons per acre but much concentrated solutions – i.e., 5 to 30 gallons per acre – are used in aerial applications. For the current risk assessment, the central value is taken as 30 gallons per acre and the range is taken as 5 to 400 gallons per acre. It should be noted that the selection of application rates and dilution volumes in this risk assessment is intended to simply reflect typical or central estimates as well as lower and upper ranges. In the assessment of specific program activities, the Forest Service will use program specific application rates in the worksheets that are included with this report to assess any potential risks for a proposed application.

The product label for Dimilin 25W specifically requires a 25 foot buffer for ground applications and a 150 foot buffer for aerial applications. These buffers indicate an area between the treated area and open bodies of water that may not be treated with diflubenzuron. The product label for Dimilin 4L does not specify a buffer but does indicate that the formulation cannot be applied to

water or *"…to areas where surface water is present"* (C&P Press 2004). In the aerial or ground applications, the USDA will use at least a 100 foot buffer and will extend the buffer up to 500 feet in some instances (Cook 2004).

2.4. Use Statistics

In order to minimize the ecological effects and human health effects of gypsy moth infestations, the USDA has adopted various intervention strategies that are roughly categorized as suppression, eradication, and slow the spread (USDA 1995). Suppression efforts are conducted by the USDA Forest Service in areas of well established gypsy moth infestations to combat or interdict periodic gypsy moth population outbreaks. Eradication efforts are conducted by USDA/APHIS to completely eliminate gypsy moth populations in areas where new populations of the gypsy moth are found. Slow the spread, as the name implies, is a program to reduce the expansion of gypsy moth populations from areas of established populations to adjacent non-infested areas.

As indicated in Table 2-3, a total of 664,560 acres were treated with diflubenzuron formulations between 1995 and 2003, for an average annual treatment of about 73,840 acres per year. Virtually all (about 99%) of this use occurred in suppression programs with only about 1% of the use slow the spread programs. Very little diflubenzuron has been used in eradication programs – i.e., only 6 acres were treated in eradication programs accounting for <0.001% of the total acres treated for suppression, eradication, and slow the spread combined. Complete statistics for the amount of diflubenzuron applied in these applications has not been encountered. At the maximum labeled rate of 0.0624 lbs a.i./acre, the average annual treatment of about 73,840 acres per year would correspond to about 4608 pounds per year.

By comparison, the annual use of diflubenzuron on cotton for 1992 (the most recent year for which statistics are available) was 78,013 lbs (USGS 1998) or about a factor of 17 above the estimated average annul use by the Forest Service. The low use of the diflubenzuron by the USDA relative to agricultural applications – i.e., about 5.6% $[4608 \div (78,013 + 4608) = 0.0558]$ – indicates that the use of diflubenzuron by the USDA will not contribute substantially to general levels of diflubenzuron in the environment. This 5.6% figure probably overestimates the use of diflubenzuron by the USDA relative to agricultural applications because USGS (1998) reports only use on cotton. Diflubenzuron is registered for application to a number of other agricultural crops. Nonetheless, localized release of diflubenzuron will occur and the consequences of this release is considered in the remainder of this risk assessment.

3. HUMAN RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION
3.1.1. Overview
No information is available on the effects of diflubenzuron on humans but the toxicity of this compound has been well characterized in experimental mammals. In mammals, the most sensitive effect involves damage to hemoglobin, a component of blood involved in the transport of oxygen. Diflubenzuron causes the formation of methemoglobin, a form of hemoglobin that is not able to transport oxygen. Methemoglobinemia, an excessive formation of methemoglobin, is the primary toxic effect of diflubenzuron by all routes of exposure and for all durations of exposure in all species of animals that have been tested. Diflubenzuron causes other effects on the blood but methemoglobinemia is the most sensitive effect – i.e., the effect that occurs at the lowest dose. While effects on the blood are well documented, there is little indication that diflubenzuron causes other specific forms of toxicity. Diflubenzuron does not appear to be neurotoxic or immunotoxic, does not appear to affect endocrine function in laboratory mammals, and is not a carcinogen. In addition, diflubenzuron does not appear to cause birth defects or reproductive effects. Diflubenzuron is relatively nontoxic by oral administration, with single-dose LD_{50} values reported as > 4640 mg/kg to $>10,000$ mg/kg. A large number of studies on the subchronic and chronic toxicity of diflubenzuron are available. As with acute toxicity, methemoglobinemia is the most consistent and sensitive sign of toxicity in laboratory mammals. Diflubenzuron can be absorbed from the skin in sufficient amounts to cause hematologic effects – e.g., methemoglobinemia and sulfhemoglobinemia. Nonetheless, these effects occur at higher doses after dermal administration than after oral administration.

3.1.2. Mechanisms of Action
Some specific mechanisms of action for diflubenzuron are well understood in both mammals and invertebrates. As discussed in Section 4.1, diflubenzuron inhibits chitin synthesis in invertebrates and this in turn disrupts normal growth and development and can lead to death. Mammals, including humans, do not produce chitin and this mechanism thus has no relevance to the human health risk assessment. Another mechanism of diflubenzuron involves damage to hemoglobin, a key component of blood, through the development of methemoglobin and sulfhemoglobin. This is highly relevant to the human health risk assessment and the formation of methemoglobin is the basis for the U.S. EPA RfD for diflubenzuron (Section 3.3).

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

common methemoglobin reductase is dependent on NADH, a molecule that is common in all living systems and is necessary for the proper function of many enzymes (Lo and Agar 1986). Some individuals are deficient in NADH-dependent methemoglobin reductase, in which case as much as 50% of their blood pigment may exist as methemoglobin. Newborns are also deficient in NADH-methemoglobin reductase. As discussed further in Section 3.1.15 (Impurities and Metabolites), 4-chloroaniline, a metabolite of diflubenzuron, has also been shown to induce methemoglobinemia (WHO 2003).

Sulfhemoglobinemia is characterized by the presence of abnormal pigments, other than methemoglobin, in red cells and can be regarded as a form of nonspecific oxidative damage (Smith 1996) and, in some cases, the differential diagnosis of sulfhemoglobinemia and methemoglobinemia may be difficult (Demedts et al. 1997). As with methemoglobinemia, sulfhemoglobinemia can be induced by aromatic amines and hydroxyamines. Unlike methemoglobinemia, sulfhemoglobinemia is irreversible. Sulfhemoglobinemia is associated with the formation of Heinz bodies, dark-staining granules found in red blood cells. The formation of Heinz bodies can lead to red cell dysfunction and hemolysis (breakdown of the cell membrane). The damaged cells are in turn captured by the spleen, which can lead to spleen enlargement. In general, cats, mice, dogs, and humans are more susceptible to Heinz body formation compared with rabbits, monkeys, chickens, and guinea pigs (Smith 1996). Studies on the effects of diflubenzuron on methemoglobin, sulfhemoglobin, Heinz body formation, and the spleen are summarized in Appendix 1. These data are discussed in further detail in Section 3.3 (Dose-Response Assessment).

While diflubenzuron displays other types of toxicity, as discussed in the following subsections, the formation of methemoglobin and sulfhemoglobin are the only mechanisms of toxicity that have been clearly identified.

3.1.3. Kinetics and Metabolism
3.1.3.1. Oral Absorption – Diflubenzuron appears to be readily absorbed after oral administration but the extent of absorption is dose-dependant. Cameron et al. (1990) conducted a standard pharmacokinetic study on diflubenzuron in rats. Diflubenzuron was rapidly absorbed and excreted in both the urine and feces. Urine showed significant levels of 2,6-difluorobenzoic acid, 2,6-difluorophippuric acid, 2,6-difluorobenzaimide, 4-chlorophenyl urea, and 2'-hydroxydiflubenzuron. Fecal excretion contained mostly unchanged parent compound. 4-Chloroaniline was not detected in urine or bile (limit of detection = 7.5 ng/mL). As discussed further below, 4-chloroaniline is a metabolite of diflubenzuron in some species (Section 3.1.3.3) and is an environmental metabolite of diflubenzuron formed by biodegradation in soil. The oral absorption of diflubenzuron appears to be dependent on dose (e.g., Willems et al. 1980). At relatively low doses, in the range of 1 mg/kg/day, a substantial fraction of administered diflubenzuron (about 50%) is absorbed. At much higher doses, in the range of 1000 mg/kg/day, much less diflubenzuron is absorbed (about 5%) (WHO 1996, 2001). While studies on the basis for this dose-dependent absorption have not been located for diflubenzuron, this is a relatively common pattern in many compounds that are highly lipophilic – i.e., tend to concentrate in fat

tissue – and probably involves saturable transport by the lymphatic system (e.g., Rozman et al. 1979).

3.1.3.2. Dermal Absorption – No studies have been found on the dermal absorption of diflubenzuron in humans. Dermal absorption in rats has been studied by Andre (1996) and this study is summarized in Appendix 1. The dermal absorption of diflubenzuron appeared to be linear for doses of 0.005 or 0.05 mg/cm^2. This is unlike the pattern with oral absorption, as noted above, but the dermal doses are very low. In addition and unlike the case with oral absorption, there is no basis for asserting that dermal absorption is saturable. Andre (1996) does not provide a kinetic analysis of the absorption data. Andre (1996) does note that about 6% of the dose was bound to skin and that less than 1% of the dose was absorbed systemically over a 10 hour period. Taking 1% as an approximate measure of absorbed dose, the dermal absorption coefficient would be about 0.001 hour^{-1} [k = -ln(1-0.01)/10 hour = 0.001 hour^{-1}].

While several additional studies are available on the toxicity of diflubenzuron after dermal administration (Section 3.1.12.), these studies do not address the kinetics of dermal absorption. WHO (1996, 2001) summarizes an unpublished study conducted in the Netherlands indicating that 0.2% of a dermal dose of 150 mg/kg was absorbed by rabbits over a 6 hour exposure period. This corresponds to a dermal absorption rate of about 0.04 hour^{-1} [k = -ln(1-0.002)/6 hours = 0.000358 hour^{-1}], substantially less than the estimate in rats from the study by Andre (1996).

Estimates of first-order dermal absorption rates can also be made from structure activity relationships (SERA 2001). Based on these relationships, the estimated first-order dermal absorption rate for diflubenzuron is 0.0044 hour^{-1} with a 95% confidence interval of 0.0019 hour^{-1} to 0.01 hour^{-1} (Worksheet A09). These estimate first-order dermal absorption rates are somewhat higher than those based on experimental measurements. The higher dermal absorption rates from Worksheet A09 are used in the current risk assessment. While this is a somewhat conservative or protective approach, it has little impact on the risk characterization (Section 3.4) because none of the exposures based on these conservative estimates approach a level on concern.

Dermal exposure scenarios involving immersion or prolonged contact with chemical solutions use Fick's first law and require an estimate of the permeability coefficient, K_p, expressed in cm/hour (SERA 2001). Using the method recommended by U.S. EPA/ORD (1992), the estimated dermal permeability coefficient for diflubenzuron is 0.012 cm/hour with a 95% confidence interval of 0.0066 to 0.021 cm/hour. The application of this method to diflubenzuron is given in Worksheet A10.

Note that the first-order and zero-order absorption coefficients are summarized in Worksheet 03 but are rounded to two significant places. Links to these values are used in all of the exposure worksheets involving dermal absorption.

3.1.3.3. Metabolism – Two types of metabolites are considered in this risk assessment: metabolites that are formed *in vivo* by an animal after diflubenzuron has been absorbed and metabolites that the formed in the environment through the degradation of diflubenzuron in environmental media – i.e., soil, air, and water. The *in vivo* metabolism of diflubenzuron has been reviewed by WHO (1996, 2001) and additional unpublished studies have been submitted to the U.S. EPA on the metabolism of diflubenzuron in rats (Cameron et al. 1990; Gay et al. 1999) as well as the environmental metabolism of diflubenzuron (Dzialo and Maynard 1999; Thus et al. 1991; Walstra and Joustra, 1990).

An overview of the *in vivo* and environmental metabolism of diflubenzuron is given in Figure 3-1. Two basic pathways exist for the metabolism of diflubenzuron. In the environment as well as in sheep, pigs, and chickens, the major route of metabolism involves cleavage of the ureido bridge with the formation of 2,6-difluorobenzoic acid and 4-chlorophenyl urea. The latter compound is then metabolized to 4-chloroaniline.   As discussed further in Section 3.1.15, the formation of 4-chloroaniline is important to the human health risk assessment because this compound is classified as a carcinogen. The other pathway for the metabolism of diflubenzuron predominates in rats and cows and involves hydroxylation rather than cleavage of the ureido bridge. Hydroxylation of the aromatic rings involves the addition of a hydrogen-oxygen or hydroxy (OH) group to one of the rings. Hydroxylation increases the water solubility of aromatic compounds. Particularly when followed by conjugation with other water soluble compounds in the body, such as sugars or amino acids, hydroxylation greatly facilitates the elimination of the compound in the urine or bile. As detailed further by WHO (2001), the ureido bridge may also be cleaved in rats but 4-chloroaniline does not appear to be a major metabolite. No information has been located on the metabolism of diflubenzuron in humans.

3.1.4. Acute Oral Toxicity

No information has been found on the acute toxicity of diflubenzuron in humans. Information regarding the acute toxicity of diflubenzuron and diflubenzuron formulations in laboratory mammals is summarized in Appendix 1. These data indicate that diflubenzuron is relatively nontoxic by oral administration, with single dose LD_{50} values in mice and rats reported as > 4640 mg/kg to >10,000 mg/kg. In other words, less than half of the animals died at these doses. Many of the exposure scenarios considered in the current risk assessment for the use of diflubenzuron for the control of the gypsy moth do involve very short term acute exposures and the use of acute oral toxicity values is considered further in Section 3.3.3.

3.1.5. Subchronic and Chronic Toxicity

No information has been found on the subchronic or chronic toxicity of diflubenzuron in humans. A large number of studies using experimental mammals are available on the subchronic and chronic toxicity of diflubenzuron. Studies most relevant to the current risk assessment as summarized in Appendix 1 and additional information, including unpublished studies conducted in Europe, are summarized by WHO (1996, 2000).

As with acute toxicity, methemoglobinemia is the most consistent and sensitive sign of toxicity in laboratory mammals and has been observed in all mammalian species on which bioassays have been conducted: cats (Keet et al. 1982), dogs (Chesterman et al. 1974; Keet et al. 1982; Greenough et al. 1985), mice (Colley et al. 1981; Colley et al. 1984; Keet et al. 1984b), rats (Berberian and Enan 1989; Burdock et al. 1980; Burdock 1984; Keet et al. 1984a), and sheep (Keet et al. 1982).

For the current risk assessment, the most relevant longer-term toxicity study is the one-year oral toxicity study in which dogs were administered diflubenzuron in gelatin capsules at doses of 0, 2, 10, 50, or 250 mg/kg/bw (Greenough et al. 1985). As indicated in Appendix 1 and discussed further in Section 3.3.2, this is the study on which the U.S. EPA (1988; 1997a; 2000) has based the chronic RfD. In this study, no clinical signs of toxicity or pathology attributable to treatment were observed. The only adverse effects that were observed included dose-related increases in methemoglobin and sulfhemoglobin accompanied by an increase in spleen weight. As noted in the previous section, the increased spleen weight is probably secondary to the hematologic effects of diflubenzuron. This study is also important in that a clear duration-response relationship is apparent, with no significant changes in methemoglobin and sulfhemoglobin concentrations at four weeks after the start of dosing.

3.1.6. Effects on Nervous System

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

Diflubenzuron, however, evidences few characteristics of a neurotoxicant even in terms of indirect effects. In an acute inhalation study involving a diflubenzuron formulation not used by the USDA (i.e, Dimilin 2L), excessive salivation and labored breathing were observed both during and after exposure (Hoffman 1997). While these can be signs of neurologic effects, they can be secondary to general irritation as well as other toxic effects. The only study on diflubenzuron that specifically assayed for neurotoxicity is the inhalation study by Newton (1999) in rats (details in Appendix 1). The neuro-behavioral battery included assays for autonomic effects, central nervous system effects (e.g., tremors and convulsions), general motor activity, movement and posture, reactivity to handling or sensory stimuli, grip strength, and observations for atypical behavior. Newton (1999) noted no treatment related effects of any endpoints assayed. The review of this study by WHO (2001) indicates that: "A reduction in 'grid count' was evident in the neuro-functional assessment of males and females exposed to 110 mg/m^3." Here, grid count refers to the number of grids that both front feet simultaneously touched during a fixed observations period. Based on the data reported in Newton (1999) for males (summary in Table 3, p. 44 and individual data in Appendix pp. 150-151 in Newton 1999)

and females (summary Table 3, p. 47 and individual data in Appendix pp. 168-169 in Newton 1999), a slight reduction in mean grid count is apparent for this response in study weeks 1, 2, and 3 but not in study week 4. There is, however, substantial scatter in the individual data in terms of the relationship of the response to concentration. The significance of the changes in grid count in the absence of any other sign of neurotoxicity is questionable.

3.1.7. Effects on Immune System

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

There is very little direct information on which to assess the immunotoxic potential of diflubenzuron. The only studies specifically related to the effects of diflubenzuron on immune function are skin sensitization studies (Section 3.1.11). While the study by Blaszcak (1997e) indicates that diflubenzuron is not a skin sensitizer, this provides no information useful for directly assessing the potential for diflubenzuron to disrupt immune function.

Nonetheless, the toxicity of diflubenzuron has been examined in numerous acute, subchronic, and chronic bioassays. Although many of these studies did not focus on the immune system, changes in the immune system (which could potentially be manifest as increased susceptibility to infection compared to controls) were not observed in any of the available long-term animal studies (Appendix 1).   Typical subchronic or chronic animal bioassays conduct morphological assessments of the major lymphoid tissues, including bone marrow, major lymph nodes, spleen and thymus (thymus weight is usually measured at autopsy as well), and blood leukocyte counts. These assessments can detect signs of inflammation or injury indicative of a direct toxic effect of the chemical on the lymphoid tissue. Changes in cellularity of lymphoid tissue and blood, indicative of a possible immune system stimulation or suppression, can also be detected (Durkin and Diamond 2002). None of these effects have been noted in any of the longer term toxicity studies on diflubenzuron (Appendix 1).

3.1.8. Effects on Endocrine Function

The *endocrine system* participates in the control of metabolism and body composition, growth and development, reproduction, and many of the numerous physiological adjustments needed to maintain constancy of the internal environment (*homeostasis*). The *endocrine system* consists of *endocrine glands*, *hormones*, and *hormone receptors*. *Endocrine glands* are specialized tissues that produce and export (*secrete*) *hormones* to the bloodstream and other tissues. The major endocrine glands in the body include the adrenal, hypothalamus, pancreas, parathyroid, pituitary,

thyroid, ovary, and testis. Hormones are also produced in the gastrointestinal tract, kidney, liver, and placenta. *Hormones* are chemicals produced in endocrine glands that bind to *hormone receptors* in target tissues. Binding of a hormone to its receptor results in a process known as *postreceptor activation* which gives rise to a *hormone response* in the target tissue, usually an adjustment in metabolism or growth of the target tissue. Examples include the release of the hormone *testosterone* from the male testis, or *estrogen* from the female ovary, which act on receptors in various tissues to stimulate growth of sexual organs and development of male and female sexual characteristics. The target of a hormone can also be an endocrine gland, in which case, receptor binding may stimulate or inhibit hormone production and secretion. Adverse effects on the endocrine system can result in abnormalities in growth and development, reproduction, body composition, homeostasis (the ability to tolerate various types of stress), and behavior.

There is no indication that diflubenzuron causes endocrine disruption in experimental mammals. Standard subchronic, chronic and reproductive toxicity studies provide no basis for asserting that any signs of overt toxicity are related to changes in endocrine function. As discussed further in Section 4, however, a few studies do indicate a potential endocrine effects in sheep (Section 4.1.2.1), birds (Section 4.1.2.2) and terrestrial insects (Section 4.1.2.3) but the strength of the association is limited.

3.1.9. Reproductive and Teratogenic Effects

Diflubenzuron has been tested for its ability to cause birth defects (i.e., teratogenicity) as well as its ability to cause reproductive and developmental impairment. Teratogenicity studies typically entail gavage administration to pregnant rats or rabbits on specific days of gestation. Two such studies (each of which is detailed in Appendix 1) were conducted on diflubenzuron: one in rats (Kavanagh 1988a) and one in rabbits (Kavanagh 1988a). As discussed by U.S. EPA/OPP (1997a), both of these were screening studies conducted at one very high dose, 1000 mg/kg bw. Since no signs of maternal or fetal toxicity were observed, no additional testing was required.

Another type of reproduction study involves exposing more than one generation of the test animal to the compound. One such study has been conducted on diflubenzuron (Brooker 1995). As detailed in Appendix 1, this study involved dietary exposures at concentrations of 0, 500, 5000, or 50,000 ppm over two generations in rats. No effects on reproductive performance were noted even though effects were seen on body weight (F_0 only) and increases were noted in methemoglobin and spleen weight – i.e., effects that may be attributable to diflubenzuron.

3.1.10 Carcinogenicity and Mutagenicity
There are no epidemiology studies or case reports that demonstrate or suggest that exposure to diflubenzuron leads to cancer in humans.

The carcinogenicity of diflubenzuron has been tested in rats and mice and these studies are detailed in Appendix 1. No carcinogenic effects were observed in rats exposed to diflubenzuron in a 2-year feeding study (Keet et al. 1984a). Neither treated nor control rats had cancers of any type, although pathological changes were observed in the spleen of both male and female rats. In mice, no carcinogenic effects or changes in spleen pathology were observed in males or females in a 2-year feeding study (Colley et al. 1984).

In addition to its lack of carcinogenic activity in *in vivo* bioassays, several bioassays of diflubenzuron for mutagenicity or other damage to DNA have failed to detect adverse effects. A lack of mutagenic activity has been reported in a dominant lethal study in mice (Arnold 1974), cell transformation assays using BALB/3T3 cells (Brusick and Weir 1977a), the induction of unscheduled DNA synthesis (Brusick and Weir 1977b), transplacental transformation assays using hamster cells (Quarles et al. 1980), and Ames assays using various strains of Salmonella typhimurium with and without metabolic activation (Brusick and Weir 1977c). Diflubenzuron did induce cell transformations in BALB/c 3T3 cells in the absence of metabolic activation; however, the effect was not observed with metabolic activation (Perocco and others 1993).

Diflubenzuron has been shown to inhibit the uptake of uridine, adenosine, and cytidine in cultured melanoma cells (Mayer et al. 1984) and inhibit the in vivo growth of melanomas in mice (Jenkins et al. 1986). Since the inhibition was enhanced by mixed function oxidase induction with 3-methylcholanthrene or beta-napthaflavone, aromatic hydroxylation was suggested as a requisite to tumor inhibition.

Both the U.S. EPA/OPP (1996a) and the WHO (1996, 2001) have concluded that diflubenzuron is not a carcinogen. This is detailed further in Section 3.3.2.3. However, the potential carcinogenicity of 4-chloroaniline, an environmental metabolite of diflubenzuron, is of concern and this is discussed further in Section 3.1.15 (Impurities and Metabolites) and in the dose-response assessment (Section 3.3.3.3).

3.1.11. Irritation and Sensitization (Effects on the Skin and Eyes)
As summarized in Appendix 1, diflubenzuron and formulations of diflubenzuron do not appear to be skin irritants (Blaszcak 1997d;) or sensitizers (Blaszcak 1997e). When instilled directly into the eye, however, diflubenzuron does cause slight to moderate conjunctival irritation (Blaszcak 1997c).

3.1.12. Systemic Toxic Effects from Dermal Exposure
As noted in Section 3.1.3.2, diflubenzuron can be absorbed from the skin and many of the exposure scenarios considered in this risk assessment involve dermal contact (Section 3.2). The available toxicity studies clearly indicate that diflubenzuron can be absorbed in sufficient

amounts to cause hematologic effects – e.g., methemoglobinemia and sulfhemoglobinemia (Goldenthal 1996).   Nonetheless, these effects occur only at higher doses after dermal administration (i.e., 1000 mg/kg/day) than after oral administration (i.e., about 100 to 250 mg/kg/day). As with oral toxicity, severe signs of dermal toxicity are not observed even at doses that will induce methemoglobinemia and sulfhemoglobinemia (Blaszcak 1997b; Goldenthal 1996). This is an important relationship that impacts that characterization of risk, as detailed further in Section 3.4.

3.1.13. Inhalation Exposure

As with oral and dermal exposure, inhalation exposures appear to primarily effect the blood, causing increases in methemoglobin and sulfhemoglobin (Eyal 1999; Hoffman 1997; Berczy et al. 1975; Newton 1999). The threshold for these effects appears to be lower in nose only exposures – i.e., an NOEC of 30 mg/m^3 with an effect level of 100 mg/m^3 in the study by Eyal (1999) – compared to whole body exposures – i.e., an NOEC of 500 mg/m^3.   It is unclear why this would be the case. In any event, as discussed further in Section 3.2, inhalation is not likely to be a significant route of exposure because of the low vapor pressure of diflubenzuron (Table 2-1) and ambient air will contain concentrations of diflubenzuron that are far below the NOEC values for nose-only exposure.

3.1.14. Inerts and Adjuvants

As noted in Section 2.2, Dimilin 4L contains petroleum oil [CAS No. 64742-46-7] and Dimilin 25W contains kaolin clay [CAS No. 1332-58-7] (C&P Press 2004). Kaolin clay is classified as a List 4a inert by the U.S. EPA (2004). This classification indicates that the product is considered as "Minimal risk inert ingredient". Petroleum oil with the CAS No. 64742-46-7 designation is classified as a List 2 inert which indicates a "Potentially Toxic Inert Ingredients/High Priority for Testing inerts". Details of these classifications may be found at: http://www.epa.gov/opprd001/inerts/lists.html.   The toxicology of petroleum oil has been reviewed in some detail by ATSDR (2003). At sufficiently high doses, some petroleum oils can cause gastrointestinal, central nervous system (CNS), and renal effects. Petroleum oils however are highly variable and it is difficult to assess the potential contribution of the petroleum oil in Dimilin 4L to the overall toxicity of the formulation.   No information on the toxicity of Dimilin 4L is included in the MSDS for this formulation (C&P Press 2004) or in the U.S. EPA RED (U.S. EPA/OPP 1997a) and no information on the toxicity of Dimilin 4L was encountered in the search of the U.S. EPA CBI files. The toxicity of Dimilin 2L (Blaszcak 1997a summarized in Appendix 1) appears to be comparable to that of Dimilin 25W (Koopman, 1977) as well as technical grade diflubenzuron (WHO 1996).

The identity of all inerts in both diflubenzuron formulations has been disclosed to the U.S. EPA (i.e., Drozdick 1998b,d; Vanstone 1998a,b,c) and this information has been reviewed as part of this risk assessment. This information, however, is protected under FIFRA (Section 10). Other than to state that no apparently hazardous materials have been identified, which is consistent with the MSDS for both Dimilin 4L and Dimilin 25W (C&P Press 2004), the information on the inerts in these formulations cannot be detailed.

3.1.15. Impurities and Metabolites

As with inerts, the impurities in formulations of diflubenzuron have been identified and disclosed to U.S. EPA (Drozdick 1998a,c,e; Van Kampen and Thus 1996; Vanstone 1998a,b,c; White 1998) and this information has been reviewed as part of this risk assessment. Again, this information is protected under FIFRA (Section 10) and cannot be disclosed in this risk assessment. Notwithstanding this limitation, the impurities that may be in diflubenzuron or formulations of diflubenzuron add relatively little uncertainty to this risk assessment. All toxicity studies summarized in Appendix 1 involved either technical grade diflubenzuron – i.e., diflubenzuron with any impurities – or the formulations which also contain the impurities. Thus, the available toxicity data should encompass the potential toxic effects of the impurities.

In terms of metabolites, the toxicity of most *in vivo* metabolites, as defined in Section 3.1.3.3, should also be encompassed by the available *in vivo* toxicity studies because these metabolites will be formed during the course of a standard *in vivo* toxicity study. This argument, however, does not hold for 4-chloroaniline for two reasons. First, as noted in Section 3.1.3.3, 4-chloroaniline does not appear to be metabolite in rodents, the species on which most toxicity studies have been conducted. Secondly, 4-chloroaniline is an environmental metabolite and is classified as a Group B2 carcinogen – i.e., indicating a probable human carcinogen following the classification of the U.S. EPA/OPP (1997a, 2000a) or a possible human carcinogen following the classification of the International Agency for Research on Cancer (IARC 1997). The carcinogenic activity of 4-chloroaniline has also been noted by WHO (2003). Consequently, potential exposures to 4-chloroaniline are quantitatively considered in the exposure assessment (Section 3.2), dose-response assessment (Section 3.3), and risk characterization (Section 3.4),

3.1.16. Toxicologic Interactions

There is no information on the interactions of diflubenzuron with other agents. Deleschuse et al. (1998) have investigated the cytotoxicity and induction of cytochromes P450 1A1/2 by insecticides in hepatic and epidermal cells. Diflubenzuron was one of the six pesticides studied and one of two that did not exert a cytotoxic effect in hepatocytes. In addition, de Sousa et al. (1997) noted a strong, dose-dependent, significant (p<0.001) induction of ethoxyresorufin O-deethylase (EROD) activity and or CYP1A1 mRNAs (5- to 7-fold greater than controls in human hepatocytes and approximately 7-fold greater than controls in rat hepatocytes). Any effect on hepatocytes and/or cytochrome P450 could impact how an organism would metabolize (either to toxicity or detoxify) a very large number of other compounds. The net effect of such interactions could be to enhance or inhibit toxicity and a more specific assessment would require data on specific combinations of other chemicals with diflubenzuron.

3.2. EXPOSURE ASSESSMENT
3.2.1. Overview.
Exposure assessments are conducted for both diflubenzuron and 4-chloroaniline. For diflubenzuron, a standard set of exposure scenarios are presented for both workers and members of the general public. As discussed in the hazard identification, concern for 4-chloroaniline arises because it is an environmental metabolite of diflubenzuron and is classified as a carcinogen. Thus, 4-chloroaniline is not a concern in worker exposure assessments because 4-chloroaniline will not be present at the time that diflubenzuron is applied. Nor is 4-chloroaniline a concern in some acute exposure scenarios for the general public such as direct spray during the application of diflubenzuron. Consequently, only a subset of the standard exposure scenarios – those associated with contaminated vegetation and contaminated water – are presented for 4-chloroaniline but these do include all standard chronic exposure scenarios, which are of greatest concern because of the potential carcinogenicity of 4-chloroaniline.

All exposure assessments are based on the maximum single application rate for diflubenzuron of 0.0625 lb/acre. This is also the maximum application rate for a single season. Assuming that diflubenzuron is applied in a single application at the maximum rate leads to the highest estimates of peak as well as longer term exposures. The consequences of using lower application rates are discussed in the risk characterization.

For workers applying diflubenzuron, three types of application methods are considered: directed ground spray, broadcast ground spray, and aerial spray. Central estimates of exposure for workers are approximately 0.0009 mg/kg/day for aerial workers, 0.0008 mg/kg/day for backpack workers and about 0.001 mg/kg/day for broadcast ground spray workers. Upper range of exposures are approximately 0.009 mg/kg/day for broadcast ground spray workers and 0.005 mg/kg/day for backpack and aerial workers. All of the accidental exposure scenarios for workers involve dermal exposures and most of these accidental exposures lead to estimates of dose that are either in the range of or substantially below the general exposure estimates for workers. The one exception involves wearing contaminated gloves for one-hour where the upper range of exposure is about 0.4 mg/kg/day.

For the general public, the range of acute exposures is from approximately 0.0000005 mg/kg associated with the lower range for the consumption of contaminated water from a stream by a child to 1.5 mg/kg associated with the upper range for consumption of contaminated fish by subsistence populations – individuals who consume free-caught fish as a major proportion of their diet. Relatively high dose estimates are also associated with the consumption of contaminated water after an accidental spill (about 0.13 mg/kg at the upper range of exposure) and for the consumption of fish by members of the general public (0.3 mg/kg). Other acute exposures are lower by about an order of magnitude or greater. For chronic or longer term exposures, the modeled exposures are much lower than for acute exposures, ranging from approximately 0.00000002 mg/kg/day (2 in 10 millionths of a mg/kg/day) associated with the lower range for the consumption of contaminated water to approximately 0.002 mg/kg/day associated with the upper range for consumption of contaminated fruit.

Exposures to 4-chloroaniline from contaminated vegetation are likely to be below corresponding exposures to diflubenzuron by a factor of about 0.02. This follows from the expected rapid dissipation of 4-chloroaniline that is derived from diflubenzuron which has been deposited on vegetation. Estimated concentrations of 4-chloroaniline in water, however, are likely to equal or exceed anticipated concentrations of diflubenzuron under some circumstances. The peak exposures to 4-chloroaniline will occur at different times (later after the application of diflubenzuron) and under different conditions of precipitation than those of diflubenzuron. These differences are due to the relatively slow rate in the formation of 4-chloroaniline from diflubenzuron in soil.

3.2.2. Workers.

The Forest Service uses a standard set of exposure assessments in all risk assessment documents. All of the exposure assessments for workers as well as members of the general public are detailed in the worksheets on diflubenzuron that accompany this risk assessment (Supplement 1) and documentation for these worksheets is given in SERA (2003). A copy of this documentation is available at www.sera-inc.com. This section on workers and the following section on the general public provide plain verbal descriptions of the worksheets and discuss diflubenzuron specific data that are used in the worksheets.

A summary of the exposure assessments for workers is presented in Worksheet E02 of the worksheets for diflubenzuron that accompany this risk assessment. Two types of exposure assessments are considered: general and accidental/incidental. The term *general* exposure assessment is used to designate those exposures that involve estimates of absorbed dose based on the handling of a specified amount of a chemical during specific types of applications. The accidental/incidental exposure scenarios involve specific types of events that could occur during any type of application. The exposure assessments developed in this section as well as other similar assessments for the general public (Section 3.2.3) are based on the maximum single and maximum annual application rate of 0.0624 lb/acre (Section 2). The consequences of using lower application rates are discussed further in the risk characterization (Section 3.4).

3.2.2.1. General Exposures – As described in SERA (2001), worker exposure rates are expressed in units of mg of absorbed dose per kilogram of body weight per pound of chemical handled. Based on analyses of several different pesticides using a variety of application methods, default exposure rates are estimated for three different types of applications: directed foliar (backpack), boom spray (hydraulic ground spray), and aerial.

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

As described in SERA (2001), worker exposure rates are expressed in units of mg of absorbed dose per kilogram of body weight per pound of chemical handled. These exposure rates are

based on worker exposure studies on nine different pesticides with molecular weights ranging from 221 to 416 and log K_{ow} values ranging from -0.75 to 6.50. The estimated exposure rates are based on estimated absorbed doses in workers as well as the amounts of the chemical handled by the workers. As summarized in Table 2-1 of this risk assessment, the molecular weight of diflubenzuron is 320 and the log K_{ow} is about 3.9. These values are within the range of the pesticides used in SERA (2001). As described in SERA (2001), the ranges of estimated occupational exposure rates vary substantially among individuals and groups, (i.e., by a factor of 50 for backpack applicators and a factor of 100 for mechanical ground sprayers). It seems that much of the variability can be attributed to the hygienic measures taken by individual workers (i.e., how careful the workers are to avoid unnecessary exposure); however, pharmacokinetic differences among individuals (i.e., how fast individuals absorb and excrete the compound) also may be important.

The number of acres treated per hour is taken from previous USDA risk assessments (USDA/FS 1989a,b,c). The number of hours worked per day is expressed as a range, the lower end of which is 6 hours based on an 8-hour work day with 1 hour at each end of the work day spent in activities that do not involve exposure to the compound. The upper end of the range, 8 hours per day, is based on an extended (10-hour) work day, allowing for 1 hour at each end of the work day to be spent in activities that do not involve exposure to the chemical.

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

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

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

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

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

As detailed in Section 3.1.3, there are various methods for estimating absorbed doses associated with accidental dermal exposure (U.S. EPA 1992a, SERA 2001). Two general types of exposure are modeled: those involving direct contact with a solution of the herbicide and those associated with accidental spills of the herbicide onto the surface of the skin. Any number of specific exposure scenarios could be developed for direct contact or accidental spills by varying the amount or concentration of the chemical on or in contact with the surface of the skin and by varying the surface area of the skin that is contaminated.

For this risk assessment, two exposure scenarios are developed for each of the two types of dermal exposure, and the estimated absorbed dose for each scenario is expressed in units of mg chemical/kg body weight. Both sets of exposure scenarios are summarize in Worksheet E01, which references other worksheets in which the specific calculations are detailed.

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

For both scenarios (the hand immersion and the contaminated glove), the assumption of zero-order absorption kinetics is appropriate. Following the general recommendations of U.S. EPA/ORD (1992), Fick's first law is used to estimate dermal exposure. As discussed in Section 3.1.3, an experimental dermal permeability coefficient (Kp) for diflubenzuron is not available. Thus, the Kp for diflubenzuron is estimated using the algorithm from U.S. EPA (1992a), which is detailed in Worksheet A10.

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

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

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

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

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

3.2.3.2. Direct Spray – Direct sprays involving ground applications are modeled in a manner similar to accidental spills for workers (Section 3.2.2.2). In other words, it is assumed that the individual is sprayed with a solution containing the compound and that an amount of the compound remains on the skin and is absorbed by first-order kinetics. For these exposure scenarios, it is assumed that during a ground application, a naked child is sprayed directly with diflubenzuron. These scenarios also assume that the child is completely covered with

diflubenzuron (that is, 100% of the surface area of the body is exposed and contaminated). These exposure scenarios are likely to represent upper limits of plausible exposure. An additional set of scenarios are included involving a young woman who is accidentally sprayed over the feet and legs. For each of these scenarios, some assumptions are made regarding the surface area of the skin and body weight, as detailed in the Series B Worksheets.

3.2.3.3. Dermal Exposure from Contaminated Vegetation – In this exposure scenario, it is assumed that the herbicide is sprayed at a given application rate and that an individual comes in contact with sprayed vegetation or other contaminated surfaces at some period after the spray operation. For these exposure scenarios, some estimates of dislodgeable residue and the rate of transfer from the contaminated vegetation to the surface of the skin must be available. No such data are available on dermal transfer rates for diflubenzuron and the estimation methods of Durkin et al. (1995) are used as defined in Worksheet D02. The exposure scenario assumes a contact period of one hour and assumes that the chemical is not effectively removed by washing for 24 hours. Other estimates used in this exposure scenario involve estimates of body weight, skin surface area, and first-order dermal absorption rates, as discussed in the previous section.

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

3.2.3.4.1. Accidental Spill – The accidental spill scenario assumes that a young child consumes contaminated water shortly after an accidental spill into a small pond. The specifics of this scenarios are given in Worksheet D05. Because this scenario is based on the assumption that exposure occurs shortly after the spill, no dissipation or degradation of diflubenzuron is considered. This scenario is dominated by arbitrary variability and the specific assumptions used will generally overestimate exposure. The actual concentrations in the water would depend heavily on the amount of compound spilled, the size of the water body into which it is spilled, the time at which water consumption occurs relative to the time of the spill, and the amount of contaminated water that is consumed. Based on the spill scenario used in this risk assessment, the concentration of diflubenzuron in a small pond is estimated to range from about 0.014 mg/L to 1.1 mg/L with a central estimate of about 0.2 mg/L (Worksheet D05). This is and is intended to be an extreme accidental exposure scenario. The purpose of this scenario is simply to suggest the intensity of measures that would need to be taken in the event of a relatively large spill of diflubenzuron into a relatively small body of water.

3.2.3.4.2. Accidental Direct Spray/drift for a Pond or Stream – These scenarios are less severe but more plausible than the accidental spill scenario described above. The U.S. EPA typically uses a two meter deep pond to develop exposure assessments (SERA 2004). If such a pond is directly sprayed with diflubenzuron at the nominal application rate of 0.0624 lb/acre, the peak concentration in the pond would be about 0.0035 mg/L (3.5 µg/L or 3.5 ppb) (Worksheet D10a). This concentration is a factor of about 300 below the peak concentration of 1.1 mg/L after the accidental spill. Because the USDA will not directly spray open bodies of water but will use buffers of 100 to 500 feet (Section 2.3), the concentration of 0.0035 mg/L from direct spray would be an accidental exposure. Using the 100 to 500 foot buffers, drift of diflubenzuron from aerial applications would result in water concentrations between about 7.7×10^{-06} mg/L (about 0.008 ppb or 8 ppt – parts per trillion) to about 6.8×10^{-05} mg/L (0.07 ppb or 70 ppt) (Worksheet 10a).

Similar calculations can be made for the direct spray of a stream and the resulting water concentrations will be dependant on the surface area of the stream that is sprayed and the rate of water flow in the stream. The stream modeled using GLEAMS (see below) is about 6 feet wide and it is assumed that the pesticide is applied along a 1038 foot length of the stream with a flow rate of 710,000 L/day. The length of 1038 feet is based on the length of a side of a square 10 ha treatment plot. At an application rate of 0.0624 lb/acre, accidental direct spray onto the surface of the stream would deposit about 4047 mg and this would result in a downstream concentration of about 0.0057 mg/L. Using a buffer of 100 feet, the drift would be a fraction of 0.0195 of the application rate (Worksheet B24) and the concentration in the stream would be about 0.00011 mg/L. Details of these and additional calculations for concentrations in stream water are given in Worksheet 10b.

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

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

For the current risk assessment, the application site was assumed to consist of a 10 hectare square area that drained directly into a small pond or stream. The chemical specific values as well as the details of the pond and stream scenarios used in the GLEAMS modeling are summarized in

Table 3-1. The GLEAMS modeling yielded estimates of runoff, sediment and percolation that were used to calculate concentrations in the stream adjacent to a treated plot, as detailed in Section 6.4 of SERA (2004). The results of the GLEAMS modeling for the small stream are summarized in Table 3-2 and the corresponding values for the small pond are summarized in Table 3-3. These estimates are expressed as both average and maximum concentrations in water. The top section of each table gives the contamination rates (WCR) – i.e., the concentration of the compound in water in units of ppb (µg/L) normalized for an application rate of 1 lb/acre. The bottom section of each table gives the estimated maximum and average concentrations adjusted for the application rate of 0.0624 lb/acre (Section 2.3).

As indicated in Table 3-2, no stream contamination is estimated in very arid regions – i.e., annual rainfall of 10 inches of less. For regions with annual rainfall rates of 15 inches or more, the modeled peak concentrations in streams range from less than 0.01 µg/L (sandy soil) to about 15 µg/L (clay soil at an annual rainfall rate of 250 inches per year). While not detailed in Table 3-2, the losses from clay are associated almost exclusively with sediment loss (about 94%), with the remaining amount due to runoff. No water contamination due to percolation is modeled. This is consistent with a large body of literature on diflubenzuron indicating that downward movement in the soil horizon is extremely limited (e.g., Sundaram and Nott 1989; WHO 1996). Even in sandy soils, where very little water contamination is anticipated, percolation accounts for only about 3% of the total loss at an annual rainfall rate of 250 inches.

Modeled concentrations in a small pond (Table 3-3) are lower than those modeled in the stream. As discussed further below, this is consistent with similar modeling conducted by Schocken et al. (2001) using PRZM/EXAMS. As with the stream modeling, no surface water contamination is expected in very arid regions. For regions with annual rainfall rates of 15 inches or more, the modeled peak concentrations in ponds range from less than 0.004 µg/L (sandy soil) to about 3 µg/L (clay soil at an annual rainfall rate of 250 inches per year).

The GLEAMS scenarios do not specifically consider the effects of accidental direct spray. As discussed above and detailed in Worksheet B06b, direct spray of a standard pond could result in peak concentrations of about 3.5 µg/L, comparable to the peak levels modeled in ponds adjacent to fields with clay soil.

As discussed in Section 3.1.15, this risk assessment is also concerned with concentrations of 4-chloroaniline that could occur in water after the application of diflubenzuron. This process was also modeled using GLEAMS as described above for diflubenzuron. As illustrated in Figure 3-1, diflubenzuron does not degrade directly to 4-chloroaniline. It is first degraded to 4-chlorophenylurea which is in turn degraded to 4-chloroaniline. For the GLEAMS modeling, however, the degradation was modeled as a one-step process, disregarding the formation of 4-chlorophenylurea. This is a conservative approach in that the formation of 4-chlorophenylurea will attenuate the formation of 4-chloroaniline. As discussed further in the risk characterization (Section 3.4), this conservative approach has no impact on the risk assessment.

The chemical specific properties for 4-chloroaniline used in the GLEAMS modeling are given in Table 3-4 and the results for the stream and pond are summarized in Tables 3-5 and 3-6, respectively. The pattern seen with 4-chloroaniline is somewhat more complex than that seen with the parent compound. For example, the average and peak concentrations of 4-chloroaniline in streams is not directly related to rainfall rates (Table 3-5). The highest peak concentration, about 2 µg/L, occurs at a rainfall rate of 100 inches per year. At a rainfall rate of 250 inches per year, the modeled peak concentration is only about 0.36 µg/L. This pattern occurs because the formation of 4-chloroaniline is more rapid in soil than in water – i.e., great microbial activity in soil. Thus, at higher rainfall rates, diflubenzuron is washed rapidly from soil and lesser amounts of 4-chloroaniline are formed. A similar pattern with respect to rainfall rates is seen in the modeling results for the pond (Table 3-6).

The temporal exposures to 4-chloroaniline will also differ from those of diflubenzuron. This is illustrated in Figure 3-2 for concentrations of diflubenzuron and 4-chloroaniline in ponds at an annual rainfall rate of 150 inches. In clay and loam soils, diflubenzuron concentrations peak after the first rainfall and then steadily decline. Concentrations of 4-chloroaniline, however, peak after about 30 to 70 days. While diflubenzuron concentrations are much higher from clay than loam because of higher runoff from clay, the peak concentrations for 4-chloroaniline are similar for both clay (0.42 µg/L) and loam (0.35 µg/L), with the peak concentration in loam soil occurring somewhat later than that in clay soil. The greatest difference between diflubenzuron and 4-chloroaniline occurs in sand. As discussed above, virtually no diflubenzuron is expected to occur in ponds with very sandy soils. This is illustrated in Figure 3-2 for an annual rainfall of 150 inches, in which the concentration of diflubenzuron in water for sand is estimated at zero over the one-year model run. Nonetheless, 4-chloroaniline as a breakdown product from diflubenzuron will form and will rapidly leach through sand. Thus, for 4-chloroaniline, the peak concentrations in the pond with sandy soil, about 1.4 µg/L, are substantially higher than the peak concentrations associated with either clay or loam soils.

3.2.3.4.4. Other Modeling Efforts – A summary of the GLEAMS modeling discussed above as well as modeling of diflubenzuron conducted for other analyses is given in Table 3-7. While some of these modeling efforts involved assumptions substantially different from the GLEAMS modeling (i.e., application rates, soil types, and rainfall patterns), the results are reasonably consistent with the above estimates of concentrations in surface waters based on GLEAMS. All of these modeling efforts used PRZM/EXAMS. As discussed in SERA (2004), PRZM (Pesticide Root Zone Model) is model used by U.S. EPA that is comparable to GLEAMS. PRZM is often linked with EXAMS (Exposure Analysis Modeling System) to estimate concentrations of pesticides in water (U.S. EPA/OPPTS 2004).

In the previous diflubenzuron risk assessment for the gypsy moth program (USDA 1995), maximum modeled concentrations at an application rate of 0.0624 lb/acre, identical to the rate used in the GLEAMS modeling, maximum concentrations in streams after direct spray were estimated at 16 ppb, very close to the estimate of 22 ppb made in the current risk assessment. Concentrations of diflubenzuron in streams associated with runoff were in the range of about 2

ppb to 13 ppb. These are very similar to the central and upper range of concentrations in streams based on the GLEAM modeling (2 ppb to 16 ppb). For open water, USDA (1995) estimated a maximum concentration of 1.22 ppb, which is only somewhat below the maximum of 3 ppb based on GLEAMS.

In the reregistration eligibility decision for diflubenzuron, U.S. EPA (1997a) modeled concentrations of diflubenzuron in surface water using Tier 2 computer models. These models are not otherwise specified in U.S. EPA (1997a). Typically, Tier 2 modeling by U.S. EPA involves PRZM/EXAMS. The U.S. EPA estimates much higher concentrations in water but this is largely due to differences in application rates. For example, at an application rate of 0.67 lb/acre, about a factor of 10 higher than the rate used with GLEAMS (0.0624 lb/acre), the U.S. EPA estimates a peak concentration of about 8.1 µg/L. Adjusting for the differences in application rate, the EPA estimate would be 0.8 µg/L [8.1 µg/L × 0.0624 lb/acre ÷ 0.67 lb/acre = 0.754 µg/L], similar to the estimates using GLEAMS with clay soil at rainfall rates of 100 to 150 inches. While the U.S. EPA (1997a) does not specify rainfall rates or soil types, Tier 2 modeling generally involves "worse case" assumptions which, in this case, would be based on high runoff soils (i.e., clay) and relatively high rainfall rates. The U.S. EPA (1997a) modeling for "Forestry" applications are specified as direct application. U.S. EPA (1997a) does not indicate the nature of the forestry application but direct spray of water does not correspond to applications for the control the gypsy moth. The concentrations modeled by U.S. EPA (1997a) of about 23 µg/L at an application rate of 0.07 lb/acre is consistent with the direct spray of a small stream modeled in this risk assessment (i.e., 22µg/L) but substantially higher than the direct spray of a pond (i.e., 3µg/L). In direct applications to shallow (1.3 to 1.7 m) ponds, Sundarum et al. (1991) monitored average day 1 concentrations in ponds of about 4 µg/L at an application rate of 70 g/ha (0.062 lb/acre), consistent with the peak concentrations in ponds discussed above (Section 3.2.3.4.3).

Harned and Relyea (1997) modeled diflubenzuron applications to a 10 ha plot after the application diflubenzuron at 350 g/ha, about a factor of 5 higher than the application rate used in the GLEAMS modeling. As with the EPA, Harned and Relyea (1997) used PRZM/EXAMS but combined these models with AgDrift. Harned and Relyea (1997) employed variable rainfall rates rather than fixed rates but the individual rainfall events varied from about 2.4 to 7.2 cm or about 1 to 2.8 inches. Based on their modeling, peak concentrations in the pond were estimated at about 1 µg/L. Correcting for the difference in application rates, their estimate of 1 µg/L would correspond to 0.2 µg/L in the GLEAMS modeling – i.e., higher by a factor of 5. As indicated in Table 3-3, concentrations estimated using GLEAMS at comparable daily rainfall events ranged from 0.2 to about 0.8 µg/L.

Schocken et al. (2001) also used AgDrift with PRZM/EXAMS to model diflubenzuron in streams and ponds beneath and adjacent to forests after an application of 0.125 lb/acre, about twice the application rate used in the GLEAMS modeling. Modeled estimates indicated that the initial concentration immediately after application should not exceed 0.255 µg/L in ponds and 0.938 µg/L in streams under the canopy. In adjacent areas, modeled estimates indicated that concentrations in ponds and streams should not exceed 0.260 µg/L and 0.856 µg/L, respectively.

The higher concentrations of diflubenzuron in streams compared to ponds is consistent with the GLEAMS modeling (Tables 3-2 and 3-3). The stream concentrations modeled by Schocken et al. (2001) of 1 µg/L are about a factor of 2 below the central estimates from GLEAMS – i.e., about 2 µg/L. This is probably due to the higher stream flow rate used by Schocken et al. (2001) – i.e., 58,320,000 L/day compared to 710,000 L/day used in the GLEAMS modeling. The peak concentrations in ponds modeled by Schocken et al. (2001), about 0.2 µg/L to 0.3 µg/L are very similar to the estimates from GLEAMS at rainfall rates of about 50 inches per year.

3.2.3.4.5. Monitoring Data – Several monitoring studies (Carr et al. 1991; Nigg and Stamper 1987; Van Den Berg 1986) are available that can be used to assess the plausibility of the modeling estimates summarized in Table 3-7. The common feature in each of these studies is that concentrations in pond and/or stream water are reported and these concentrations can be associated with a defined application rate. The study by Van Den Berg (1986) is probably the most directly relevant to this risk assessment. In this study, diflubenzuron was applied to a 10-acre mixed hardwood-conifer forested plot at an application rate of 0.0625 lb/acre. Initial concentrations of diflubenzuron in surface water (streams and stream pools) in treatment area ranged from 0.127-0.203 ppb and declined to 0.029-0.045 ppb after one day. These concentrations are in the range of concentrations modeled using GLEAMS for ponds (central range) and streams (lower range). Similar results are reported by Carr et al. (1991) who monitored concentrations in streams below 0.5 ppb after the application of diflubenzuron at rates of 13 g/ha or 26 g/ha. Adjusted for an application rate of 0.0624 lb/acre (70 g/ha), the concentration of 0.5 ppb would correspond to about 2.5 to 5 ppb, very close to the upper range of stream concentrations modeled using GLEAMS. The study by Nigg and Stamper (1987) involved a very high application rate, 560 g/ha (226 g/ac or 0.5 lb/acre) in a citrus grove. The maximum monitored concentration in an adjacent pond was 0.197 ppb. Adjusted to an application rate of 0.0624 lb/acre (70 g/ha), this corresponds to a concentration of about 0.02 ppb, in the lower range of pond concentrations modeled using GLEAMS.

This discussion of the monitoring data is not intended to imply a validation of the GLEAMS modeling or other modeling efforts. Model validation or calibration can only be done on a site-specific basis. Nonetheless, the monitoring data do suggest that estimates from GLEAMS as well as other comparable modeling efforts are at least plausible and may reasonably reflect the highly variable concentrations of diflubenzuron that may occur in surface water over a wide range of site-specific conditions.

3.2.3.4.6. Concentrations of Diflubenzuron in Water Used for Risk Assessment – A summary of the concentrations of diflubenzuron in water that are used for the current risk assessment is given in Table 3-8. The upper range of the expected peak concentration of diflubenzuron in surface water will be taken as 16 µg/L for an application rate of 0.0624 lb/acre. This is based on the upper range of concentrations estimated in streams from the GLEAMS modeling. This estimate is consistent with both the available monitoring data (Section 3.2.3.4.6) and other comparable modeling efforts (Section 3.2.3.4.5). This concentration also encompasses accidental direct sprays of both a small stream and small pond (Table 3-7). In most instances,

concentrations in surface water are likely to be much lower. At the lower extreme, an argument may be made that concentrations of diflubenzuron are likely to be essentially zero – i.e., applications made at sites that are distant from open bodies of water and in areas in which runoff or percolation are not likely to occur. For this risk assessment, the lower concentration in ambient water will be set at 0.01 µg/L. This is in the lower range of non-zero concentrations modeled in streams and ponds in relatively arid regions. The central estimate of the concentration of diflubenzuron in surface water will be taken as 0.4 µg/L. This is the geometric mean of the range of 0.01 µg/L to 16 µg/L.

Longer term concentrations of diflubenzuron in surface water will be much lower than peak concentrations. At an application rate of 0.0624 lb/acre, the highest longer term concentration will be taken as 0.1 µg/L. This is near the maximum longer term concentration given by U.S. EPA (1997a) after adjusting for differences in application rate – i.e., 0.74 µg/L ÷ 6 applications at 0.06 lb/acre. This longer term maximum concentration is also near the upper range of the longer term concentrations modeled using GLEAMS – i.e., 0.06 µg/L in streams at an application rate of 0.0624 lb/acre. As with peak concentrations, the lower range of longer term concentrations will approach zero. For this risk assessment, the lower range of longer term concentrations is taken as 0.001 µg/L, the lowest non-zero value modeled for diflubenzuron in ponds. This lower range is somewhat arbitrary but has no impact on the risk assessment. The central value for longer term concentrations of diflubenzuron in water will be taken as 0.02 µg/L. This is adapted from the longer term concentrations modeled by Harned and Relyea (1997) but adjusted for differences in the application rate – i.e., 0.1 µg/L × (70 g/ha ÷ 350 g/ha) = 0.02 µg/L. This value is similar to the central estimates of longer term concentrations in streams modeled using GLEAMS – i.e., 0.01 µg/L in Table 3-7 – but is near the upper range of concentrations that would be expected in ponds – i.e., 0.06 µg/L in Table 3-7.

3.2.3.4.7. Concentrations of 4-Chloroaniline in Water Used for Risk Assessment – A summary of the concentrations of 4-chloroaniline in water that are used for the current risk assessment is given in Table 3-9. The upper range of the expected peak concentration of 4-chloroaniline in surface water will be taken as 3 µg/L for an application rate of 0.0624 lb/acre. This is based on the upper range of concentrations estimated in streams near application sites with sandy soil over a range of annual rainfall rates from about 25 to 250 inches (Table 3-5). This concentration is higher than concentrations that might be expected in ponds by about a factor of 3 (Table 3-6). As with diflubenzuron, the lower range of concentrations of 4-chloroaniline in water will approach zero. For this risk assessment, the lower range is taken as 0.00003 µg/L, the lowest non-zero concentration modeled in ponds (i.e., Table 3-6, peak concentration for loam at an annual rainfall rate of 15 inches). The central estimate is taken as 0.5 µg/L. This is about the concentration modeled in stream with loam soil over a range of annual rainfall rates of 100 to 250 inches.

Longer term concentrations of 4-chloroaniline are taken as 0.05 µg/L with a range of 0.0002 µg/L to 0.2 µg/L at an application rate of 0.0624 lb/acre. The lower range is based on the lowest non-zero concentration modeled in ponds – i.e., loam soil at an annual rainfall rate of 15 inches.

The upper range is taken as the highest concentration modeled in ponds – i.e., sandy soil at annual rainfall rate of about 25 to 100 inches. The central estimate is based on the relatively narrow range of concentrations modeled in ponds with loam soil over rainfall rates of 50 to 250 inches per year – i.e., about 0.04 to 0.06 µg/L in Table 3-6. Much lower concentrations are likely to be seen in streams.

3.2.3.5. Oral Exposure from Contaminated Fish – Many chemicals may be concentrated or partitioned from water into the tissues of animals or plants in the water. This process is referred to as bioconcentration. Generally, bioconcentration is measured as the ratio of the concentration in the organism to the concentration in the water. For example, if the concentration in the organism is 5 mg/kg and the concentration in the water is 1 mg/L, the bioconcentration factor (BCF) is 5 L/kg [5 mg/kg ÷ 1 mg/L]. As with most absorption processes, bioconcentration depends initially on the duration of exposure but eventually reaches steady state. Details regarding the relationship of bioconcentration factor to standard pharmacokinetic principles are provided in Calabrese and Baldwin (1993).

Burgess (1989) assayed the bioconcentration of diflubenzuron in Bluegill sunfish, *Lepomis macrochirus*, over a 28 day exposure using C^{14}-labeled diflubenzuron. In this study, concentrations in water, whole fish, fillet (muscle), and viscera were measured at day 0.17 (4 hours), as well as on days 1, 3, 7, 14, 21, and 28. In fillet, the fish muscle ,the BCF was 120 after 1 day and 170 after 28 days with a peak of 200 after 7 days. In whole fish, the BCF was 260 after 1 day and 350 after 28 days with a peak of 360 after 7 days. Similar BCF values have been noted for diflubenzuron by Schaefer et al. (1979, 1980).

For the human health risk assessment of diflubenzuron, the BCF in fillet of 120 after 1 day will be used for acute exposures and the maximum BCF in fillet of 200 will be used for longer term exposures. This approach is taken under the assumption that humans will consume only the fish muscle. In the ecological risk assessment, however, the assumption will be made a predatory consumes the entire fish. Thus, for the ecological risk assessment, the whole body BCF values will be used, 260 for acute exposures and 360 for longer term exposures. These values are entered into Worksheet A02 for diflubenzuron and used in the subsequent worksheets involving exposures to contaminated fish.

Less detailed information is available on the bioconcentration of 4-chloroaniline. Because 4-chloroaniline is much more water soluble than diflubenzuron and has a much lower octanol-water partition coefficient, very little bioconcentration is expected in fillet or whole fish (WHO 2003). In a 14-day exposure of carp to two concentrations of 4-chloroaniline, Tsuda et al. (1993) noted essentially no bioconcentration – i.e., the concentrations in water were essentially identical to those in the fish. Thus, in Worksheet A02 for 4-chloroaniline, values of 1 are used for all BCF values – acute and chronic, whole fish and muscle.

For all scenarios involving the consumption of contaminated fish, concentrations of diflubenzuron or 4-chloroaniline in water are identical to the concentrations used in the

contaminated water scenarios (see Section 3.2.3.4). The acute exposure scenario is based on the assumption that an adult angler consumes fish taken from contaminated water shortly after an accidental spill of 200 gallons of a field solution into a pond that has an average depth of 1 m and a surface area of 1000 m^2 or about one-quarter acre. No dissipation or degradation is considered. Because of the available and well-documented information and substantial differences in the amount of caught fish consumed by the general public and native American subsistence populations, separate exposure estimates are made for these two groups (Worksheets D08a and D08b). The chronic exposure scenario is constructed in a similar way, as detailed in Worksheets D09a and D09b, except that estimates of concentrations in ambient water are based on the longer-term estimates summarized in Table 3-8 for diflubenzuron and Table 3-9 for 4-chloroaniline.

3.2.3.6. Oral Exposure from Contaminated Vegetation – Although Forest Service applications of diflubenzuron will not involve the intentional treatment of food crops, incidental exposure to vegetation that may be consumed by members of the general public is plausible during broadcast applications. Any number of scenarios could be developed involving either accidental spraying of crops or the spraying of edible wild vegetation, like berries. The two exposure scenarios developed for this exposure assessment include one scenario for acute exposure, as defined in Worksheet D03 and one scenario for longer-term exposure, as defined in Worksheet D04. In both scenarios, the concentration of diflubenzuron on contaminated vegetation is estimated using the empirical relationships between application rate and concentration on vegetation developed by Fletcher et al. (1994) which is in turn based on a re-analysis of data from Hoerger and Kenaga (1972). These relationships are defined in Worksheet B21. For the acute exposure scenario, the estimated residue level is taken as the product of the application rate and the residue rate (Worksheet D03).

For the longer-term exposure scenario (Worksheet D04), a duration of 90 days is used. The rate of decrease in the residues over time is taken from the vegetation half-time of 9.3 days (Table 2-1). Although the duration of exposure of 90 days is somewhat arbitrary, this duration is intended to represent the consumption of contaminated fruit that might be available over one season. Longer durations could be used for certain kinds of vegetation but would lower the estimated dose (i.e., would reduce the estimate of risk).

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

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

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

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

A somewhat different approach is required to assess exposures to 4-chloroaniline. Immediately after application, residues on vegetation will be comprised solely of diflubenzuron. As diflubenzuron degrades, 4-chloroaniline may be formed. Field studies, however, have indicated no residues of 4-chloroaniline on vegetation treated with diflubenzuron (Schroeder 1980). This may be due to the rapid atmospheric degradation of 4-chloroaniline in air – i.e., an estimated halftime of 3.9 hours or about 0.16 days. This is much less than the estimated vegetation halftime for diflubenzuron – i.e., 9.3 days (Sundaram 1986, 1996). Thus, the rate limiting step in the residues of 4-chloroaniline on vegetation will be the formation of 4-chloroaniline.

The approach for estimating concentrations of 4-chloroaniline on vegetation is conceptually similar to the approach taken with estimating concentrations in water. As a simplifying assumption, 4-chloroaniline generation will be estimated from the halftime of 9.3 days of diflubenzuron – i.e., direct breakdown from diflubenzuron to 4-chloroaniline. In addition, the dissipation of 4-chloroaniline from vegetation will be taken as the atmospheric halftime of 0.16 days, from WHO (2003). Under these conditions and at steady state, the ratio of 4-chloroaniline to diflubenzuron will be ratio of the these halftimes – i.e., 0.16 days ÷ 9.3 days = 0.017. In the scenario specific worksheets for 4-chloroaniline, all specific worksheets modeling exposure to contaminated vegetation are based on concentrations of diflubenzuron. The adjustment factor of 0.017 for 4-chloroaniline is incorporated into all worksheets involving exposure to contaminated vegetation (Worksheets D03, D04, F04a, F04b, F10, F11a, F11b, F12, F13a, F13b, F14a, and F14b).

3.3. DOSE-RESPONSE ASSESSMENT
3.3.1. Overview
The dose-response assessment considers both diflubenzuron itself as well as 4-chloroaniline as an environmental metabolite of diflubenzuron. For systemic toxicity, the dose-response assessment involves the adoption or derivation of acute and chronic RfDs, doses that are considered to produce no adverse effects, even in sensitive individuals. RfDs are presented for both diflubenzuron and 4-chloroaniline. Cancer risk is considered quantitatively for 4-chloroaniline and is expressed as a dose associated with a risk of 1 in 1-million. Following standard practices for USDA risk assessments, risk assessment values available from U.S. EPA are adopted directly unless there is a compelling basis for doing otherwise. When risk values are not available from U.S. EPA, the methods used by U.S. EPA are employed to derive surrogate values.

U.S. EPA has derived a chronic RfD for diflubenzuron of 0.02 mg/kg/day. This chronic RfD is well-documented and is used directly for all longer term exposures to diflubenzuron. This value is based on a NOAEL of 2 mg/kg/day in dogs and an uncertainty factor of 100 – a factor of 10 for interspecies differences and a factor of 10 for sensitive subgroups. Because of the low acute toxicity of diflubenzuron, the U.S. EPA has not derived an acute RfD but has identified an acute NOAEL of 10,000 mg/kg. While this NOAEL could be used to derive a surrogate acute RfD of 100 mg/kg, a more conservative approach is taken and a surrogate acute RfD of 11 mg/kg is derived based on a NOAEL of 1118 mg/kg from a study using a petroleum-based formulation of diflubenzuron. Diflubenzuron has been classified as a non-carcinogen by both U.S. EPA and WHO and no quantitative cancer risk assessment for exposures to diflubenzuron is conducted.

The U.S. EPA has derived a chronic RfD for 4-chloroaniline of 0.004 mg/kg/day and this value is used in the current risk assessment to characterize risks from 4-chloroaniline for longer term exposures. This RfD is based on a chronic oral LOAEL of 12.5 mg/kg/day using an uncertainty factor of 3000, three factors of 10 for interspecies extrapolation, sensitive subgroups, and the use of a LOAEL with an additional factor of 3 due to the lack of data reproductive toxicity data. As with diflubenzuron, the U.S. EPA has not derived an acute RfD for 4-chloroaniline. For this risk assessment a conservative approach is taken in which a surrogate acute RfD of 0.03 mg/kg is based on a subchronic (90-day) NOAEL of 8 mg/kg/day. Consistent with the approach taken by U.S. EPA for the chronic RfD, an uncertainty factor of 300 is used – a factor of 10 for interspecies extrapolation, 10 for intraspecies extrapolation, and 3 for the lack of data on reproductive toxicity. For cancer risk, the U.S. EPA proposes a human cancer potency factor for 4-chloroaniline of 0.0638 $(mg/kg/day)^{-1}$. This potency factor is used to calculate a dose of 1.6×10^{-5} mg/kg/day that would be associated with a cancer risk of 1 in 1-million.

3.3.2. Diflubenzuron
3.3.2.1. Chronic RfD – The most recent RfD for diflubenzuron is 0.02 mg/kg/day. This value is given on the U.S. EPA's agency-wide list of approved RfDs (i.e., IRIS) (U.S. EPA 1990) and has been adopted by the U.S. EPA's Office of Pesticides (U.S. EPA/OPP 1997a,b, 2001a).

The chronic RfD is based on a study by Greenough et al. (1985) in which technical grade diflubenzuron was administered daily in gelatin capsules to dogs at doses of 0, 2, 10, 50, or 250 mg/kg/day, 7 days/week, for 52 consecutive weeks. At the lowest dose, 2 mg/kg/day, no effects were noted on methemoglobin formation or other standard endpoints. This study is detailed further in Appendix 1. The RfD was calculated by dividing the NOAEL of 2 mg/kg/day by an uncertainty factor of 100, a factor of 10 for interspecies differences – i.e., extrapolation of animal data to humans – and a factor of 10 for intraspecies variability – i.e., individuals who might be most sensitive to diflubenzuron.

Under the Food Quality Protection Act (FQPA), the U.S. EPA is required to consider an additional uncertainty factor of 10 for the protection of infants and children. For diflubenzuron, the U.S. EPA (1997a) determined that the additional uncertainty factor is not required because of the information on the reproductive toxicity of diflubenzuron is adequate. As discussed in Section 3.1.9, diflubenzuron has been tested for and does not appear to cause birth defects or reproductive and developmental impairment.

For this risk assessment, the chronic RfD of 0.02 mg/kg/day is used to characterize risks for the general public as well as workers in longer term exposures. Because the RfD is intended to protect for lifetime exposures, it provides a conservative basis for comparing estimated exposure levels to an index of acceptable exposure.

3.3.2.2. Acute RfD – The U.S. EPA (1997a) did not specifically derive an acute RfD for diflubenzuron. In discussing the acute oral toxicity of diflubenzuron and referring specifically to the NOAEL of 10,000 mg diflubenzuron/kg bw from the single dose study in rats and mice by Koopman (1977) – i.e., a dose of 40,000 mg Dimilim/kg bw – the U.S. EPA/OPP (1996) concludes that:

> *One day single dose oral studies in rats and mice indicated*
> *only marginal effects on methemoglobin levels at a dose level*
> *of 10,000 mg/kg of a 25% wettable powder formulation.*
> *Sulfhemoglobin levels and Heinz bodies were not affected.*
> *Therefore, there is no acute dietary endpoint and a risk*
> *assessment for acute dietary exposure (1 day) is not necessary.*
> (U.S. EPA/OPP, 1996a, p. 16).

While this is a reasonable position, the current risk assessment is concerned with characterizing the risks of acute exposures as well as comparing the risks of acute exposures to diflubenzuron with risks associated with acute exposures other agents used to control the gypsy moth. A surrogate acute RfD of 100 mg/kg could be derived using the NOAEL of 10,000 mg/kg identified by U.S. EPA/OPP (1996a) and the uncertainty factor of 100 used by U.S. EPA/OPP (1996a) in deriving the chronic RfD (Section 3.3.2.1).

A more conservative approach, however, is taken for the current risk assessment. As noted in the hazard identification (Section 3.1.14), Dimilin 4L contains petroleum oil, a substance that is considered potentially toxic. While no acute toxicity studies have been encountered on Dimilin 4L, Blaszcak (1997a) has conducted a single dose gavage study in rats with Dimilin 2L, another petroleum based formulation of diflubenzuron. In this study, no signs of toxicity associated with treatment were noted at a dose of 5000 mg/kg as Dimilin 2L, equivalent to 1118 mg/kg as diflubenzuron. Thus, 1118 mg/kg rather than 10,000 mg/kg will be taken as the acute NOAEL. This value is used to calculate an acute RfD of 11 mg/kg by applying an uncertainty factor of 100, as in the chronic RfD, and rounding to the nearest integer.

3.3.2.3. Cancer Potency – The U.S. EPA/OPP (1996a) has determined that diflubenzuron itself does not pose a carcinogenic risk. Specifically, the U.S. EPA/OPP (1997a) has concluded that:

> *Based on the available evidence, which included adequate*
> *carcinogenicity studies in rats and mice and a battery of*
> *negative mutagenicity studies, diflubenzuron per se is classified*
> *as Group E (evidence of non-carcinogenicity for humans). –*
> (U.S. EPA 1997a, p. 18)

Thus, there is no basis for identifying carcinogenicity as and endpoint of concern and this effect is not treated quantitatively in the current risk assessment. This is consistent with the evaluation of the available data on carcinogenicity by WHO (1996, 2001).

3.3.3. 4-Chloroaniline
3.3.3.1. Chronic RfD – The chronic RfD for 4-chloroaniline is 0.004 mg/kg/day (U.S. EPA 1995). This RfD is based on a 2-year feeding study using rats in which the formation of non-neoplastic lesions of the splenic capsule was observed at 250 ppm in the diet (12.5 mg/kg/day) (NCI 1979). This dose is classified as a LOAEL and is divided by an uncertainty factor of 3,000 to derive the RfD. This uncertainty factor is intended to account for intra- and interspecies differences and the extrapolation from a LOAEL to a NOAEL. A value of ten is used for each of these three uncertainty factors is given – i.e., $10 \times 10 \times 10$. An additional factor of 3 was incorporated into the uncertainty factor because of the lack of supporting reproductive toxicity data. This data gap has also been noted by WHO (2003). Confidence in the principal study, the database for toxic effects, and the RfD itself is low (U.S. EPA 1995).

For this risk assessment, the chronic RfD derived by U.S. EPA (1995) is used for characterizing longer-term risks for the general public. As with the RfD for diflubenzuron, this provides a conservative basis for assessing the risks of longer term exposures, which are typically over periods far less than lifetime.

3.3.3.2. Acute RfD – As with diflubenzuron, the U.S. EPA has not proposed an acute RfD for 4-chloroaniline. As noted in Section 3.1, acute exposures to 4-chloroaniline are likely to be minimal immediately after the application of diflubenzuron – i.e., prior to the environmental

metabolism of diflubenzuron to 4-chloroaniline. Nonetheless, as detailed in Section 3.2.3.4 and illustrated in Figure 3-2, peak exposures to 4-chloroaniline in water may be higher than peak exposures to diflubenzuron in water, although the peak 4-chloroaniline exposures may occur weeks to months after the application of diflubenzuron. Consequently, this risk assessment will derive a surrogate acute RfD for 4-chloroaniline.

The toxicology of 4-chloroaniline has been reviewed in detail by WHO (2003) and the most relevant studies for the current risk assessment as summarized in Appendix 1. As a conservative approach, the surrogate acute RfD is based on the subchronic study by Scott and Eccleston (1967) in which rats were dosed daily with 4-chloroaniline at 0, 8.0, 20.0, or 50.0 mg/kg for 3 months. No hematologic or other adverse effects were observed at the lowest dose, 8 mg/kg/day. For the surrogate acute RfD, an uncertainty factor of 300 is used – a factor of 10 for interspecies extrapolation, 10 for intraspecies extrapolation, and 3 for the lack of data on reproductive toxicity. Thus, the surrogate acute RfD is taken as 0.03 mg/kg/day [8 mg/kg/day ÷ 300 = 0.02666 mg/kg/day which rounds to 0.03 mg/kg/day using one significant figure].

3.3.3.3. Cancer Potency – In the previous risk assessment for the use of diflubenzuron in gypsy moth programs (USDA 1995), a cancer potency factor of 0.013 (mg/kg/day)$^{-1}$ was used in the human health risk assessment. This was based on the NCI (1979) using the linearized multi-stage model. More recently, the U.S. EPA/OPP (1999, 2000a) has calculated a human cancer potency factor for 4-chloroaniline of 0.0638 (mg/kg/day)$^{-1}$, about a factor of 5 greater than the previous value used by USDA (1995).

In implementing the dietary risk assessment for the formation 4-chloroaniline from diflubenzuron, the U.S. EPA (2000a) has noted a potential cancer risk from 4-chlorophenylurea. As noted in Figure 3-1 and discussed in Section 3.1.3.3, 4-chlorophenylurea is structurally similar to 4-chloroaniline and is formed as an intermediate in the environmental breakdown of diflubenzuron to 4-chloroaniline. No specific information is available on the carcinogenicity of 4-chlorophenylurea. As a conservative approach in their dietary risk assessment of the degradation products of diflubenzuron, the U.S. EPA (2000a) elected to treat 4-chlorophenylurea as if it were a carcinogen with the same potency as 4-chloroaniline. This approach has been criticized by Cardona (1999, 2001) both because of the lack of information indicating that 4-chlorophenylurea is carcinogenic and because 4-chloroaniline does not appear to be an *in vivo* metabolite of 4-chlorophenylurea in rodents.

As detailed in Section 3.2.3.4.3 for drinking water and Section 3.2.3.6 for contaminated vegetation, the current risk assessment takes a somewhat different approach to the risks posed by 4-chlorophenylurea. There is no doubt that 4-chlorophenylurea is metabolized to 4-chloroaniline in the environment. Because the toxicity data on 4-chlorophenylurea are limited, the current risk assessment models the degradation of diflubenzuron to 4-chloroaniline as a one-step process, omitting the formation of 4-chlorophenylurea. While this is conceptually different from the equal potency assumption used by U.S. EPA (2000a), it is a conservative approach but avoids the

use of a surrogate potency parameter for a compound, 4-chlorophenylurea, for which there is no evidence of carcinogenicity.

For this risk assessment, the human cancer potency factor for 4-chloroaniline of 0.0638 $(mg/kg/day)^{-1}$ proposed by U.S. EPA/OPP (1999, 2000a) is used to assess cancer risks for all longer term exposure scenarios. This potency factor is not applied directly to any acute exposure assessments. Nonetheless, it is worth noting that all of the longer term estimates of exposure are based on average values that include short-term peak exposures. Thus, these higher but transient acute exposures are incorporated into the cancer risk assessment.

In the risk characterization worksheet for 4-chloroaniline (Worksheet E04 in Supplement 2), cancer risk is expressed as the ratio of exposure (dose in mg/kg/day) to a dose with a risk of 1 in 1-million. In a linear cancer model, such as that used by U.S. EPA, risk is assumed to be linearly related to dose:

$$\text{Risk} = \text{dose} \times \text{potency}$$

Thus, taking the potency factor of 0.0638 $(mg/kg/day)^{-1}$ and a risk level of 1 in 1-million (1×10^{-6}), the dose associated with a risk of 1 in 1-million can be calculated as:

$$\text{dose} = 1 \times 10^{-6} \div 0.0638 \ (mg/kg/day)^{-1} = 0.000015673 \approx 1.6 \times 10^{-5} \ mg/kg/day$$

This dose is used in the Worksheet E04 for the risk characterization of cancer risks associated with exposure to 4-chloroaniline.

3.4. RISK CHARACTERIZATION
3.4.1. Overview
The risk characterization for potential human health effects associated with the use of diflubenzuron in USDA programs to control the gypsy moth is relatively unambiguous: none of the hazard quotients reach a level of concern at the highest application rate that could be used in USDA programs. In that many of the exposure assessments involve very conservative assumptions – i.e., assumptions that will tend to overestimate exposure – and because the dose-response assessment is based on similarly protective assumptions, there is no basis for asserting that this use of diflubenzuron poses a hazard to human health.

Notwithstanding the above assertion, it is worth noting that the greatest relative concern is with the contamination of water with 4-chloroaniline rather than with any exposures to diflubenzuron itself. The highest hazard quotient for diflubenzuron is 0.1, a factor of 10 below a level of concern. Since this hazard quotient is based on toxicity, an endpoint that is considered to have a population threshold, the assertion can be made that risk associated with exposure to diflubenzuron is essentially zero.

This is not the case with 4-chloroaniline, which is classified as a probable human carcinogen and is an environmental metabolite of diflubenzuron. For 4-chloroaniline, the highest hazard quotient is 0.4, below the level of concern by a factor of only 2.5. The scenario of greatest concern involves cancer risk from drinking contaminated water. This risk would be most plausible in areas with sandy soil and annual rainfall rates of about 50 to 250 inches. The central estimate of the hazard quotient for the consumption of water contaminated with 4-chloroaniline and based on a cancer risk of 1 in 1-million is 0.09, below the level of concern by a factor of 10.

3.4.2. Workers
A quantitative summary of the risk characterization for workers is presented in Worksheet E02 of the diflubenzuron worksheets (Supplement 1). The quantitative risk characterization is expressed as the hazard quotient, which is the ratio of the estimated exposure from Worksheet E01 to the RfD. For acute accidental/incidental exposures, the surrogate acute RfD of 11 mg/kg is used (Section 3.3.3.2). For longer term general exposures – i.e., exposures that could occur over the course of several days, weeks, or months during an application season – the chronic RfD of 0.02 mg/kg/day is used (Section 3.3.3.1).

The qualitative risk characterization for workers is reasonably unequivocal. None of the acute or longer term hazard quotients exceed 1, the level of concern. In the normal application of diflubenzuron over the course of a season or even several years, the hazard quotients range from 0.04 to 0.07 – i.e., below the level of concern by factors of about 14 to 25. At the upper ranges of exposure for workers, the hazard quotients approach but do not exceed a level of concern – i.e., 0.2 to 0.5. Similarly, the upper range of hazard quotients for accidental/incidental exposures range from 0.0001 to 0.03, below the level of concern by factors of about 33 to 10,000. As noted in Section 3.2.2.2, the only accidental/incidental exposure that exceeds general exposures involves wearing contaminated gloves for 1 hour. While the hazard quotient of 0.03 is

substantially below a level of concern, the use of contaminated gloves appears to be the greatest source of concern in the handling of diflubenzuron.

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

3.4.3. General Public
3.4.3.1. Diflubenzuron – A quantitative summary of the risk characterization for members of the general public is presented in Worksheet E04 of the diflubenzuron worksheets (Supplement 1). As with the risk characterization for workers, risk is expressed quantitatively as the hazard quotient using the surrogate acute RfD of 11 mg/kg (Section 3.3.3.2) and the chronic RfD of 0.02 mg/kg/day is used (Section 3.3.3.1).

Also as with workers, the qualitative risk characterization for members of the general public is unambiguous, with none of the acute or longer term hazard quotients exceeding 1 even at the upper ranges of plausible exposure. The highest hazard quotient is 0.1, the upper range of risk for the consumption of contaminated fish by subsistence populations. Nonetheless, this extreme acute scenario is below the level of concern by a factor of 10. No other acute exposure scenarios, many of which involve extremely conservative assumptions, approach a level of concern at the upper range of exposure. Based on central estimates of acute exposure, which involve somewhat less conservative assumptions, the acute hazard quotients range from 0.000003 to 0.02 – i.e., below the level of concern by factors of 50 to over 300,000.

3.4.3.2. 4-Chloroaniline – A quantitative summary of the risk characterization for members of the general public is presented in Worksheet E04 of the 4-chloroaniline worksheets (Supplement 2). Risk is expressed quantitatively as the hazard quotient using the surrogate acute RfD of 0.03 mg/kg (Section 3.3.3.2) and the chronic RfD of 0.004 mg/kg/day is used (Section 3.3.3.1).

In terms of both toxicity and carcinogenicity, the hazard quotients for members of the general public are comparable to but somewhat higher than the corresponding hazard quotients for diflubenzuron – a maximum hazard of 0.4 for 4-chloroaniline compared to a maximum hazard quotient of 0.1 for diflubenzuron.

The hazard quotient of 0.4 for 4-chloroaniline is associated with contamination of water, the hazard quotient for toxicity for the consumption of contaminated fish by subsistence populations and the hazard quotient for the dose associated with a cancer risk of 1 in 1-million for the longer term consumption of contaminated water. As detailed in Section 3.2.3.4 and illustrated in Figure 3-2, these risks are associated with the application of diflubenzuron to sandy soils in areas with annual rainfall rates of about 50 to 250 inches. In areas with predominantly clay or loam

soils, risks will be less by factors of about 3 to 10 (Table 3-6). Also, the relatively high hazard quotient of 0.4 is associated with standing bodies of water – i.e., ponds or lakes. Concentrations of 4-chloroaniline in streams even with sandy soil will be much less (Table 3-5).

Based on central estimates of exposure, acute hazard quotients range from 0.0004 to 0.01, below the level of concern by factors of 100 to 2500. Most chronic hazard quotients are in the range of 0.000002 to 0.0005, far below a level of concern. The only exception is the central estimate of the hazard quotient for the consumption of contaminated water based on a cancer risk of 1 in 1-million. This hazard quotient is 0.09, below the level of concern by a about a factor of 10. Nonetheless, the consumption of water that is contaminated with 4-chloroaniline as the greatest source of concern for members of the general public in the application of diflubenzuron to control the gypsy moth.

3.4.4. Sensitive Subgroups
Some individuals are born with a form of congenital methemoglobinemia and may be at increased risk of adverse effects to compounds that induce methemoglobinemia (Barretto et al. 1984). Infants less than 3 months old have lower levels of methemoglobin (cytochrome b5) reductase and higher levels of methemoglobin (1.32%), compared with older children or adults (Centa et al. 1985; Khakoo et al. 1993; Nilsson et al. 1990). A similar pattern is seen in many species of mammals (Lo and Agar 1986). Some infants with an intolerance to cow's milk or soy protein exhibit methemoglobinemia (Murray and Christie 1993; Wirth and Vogel 1988). These infants would be at increased risk if exposed to any materials contaminated with diflubenzuron or any compound that induces methemoglobinemia.

Individuals with poor diets may be at increased risk to some chemicals. Based on a study in rats (Hagler et al. 1981), iron deficiency leads to anemia but does not influence methemoglobin reductase activity. Thus, although individuals with poor nutritional status are generally a group for which there is particular concern, the available information does not support an increased concern for these individuals with respect to diflubenzuron exposure.

The RfDs used in the current risk assessment quantitatively consider sensitive subgroups. As noted in Section 3.3.2, the chronic RfD derived by U.S. EPA (1997a) incorporates a factor of 10 into overall uncertainty factor of 100 used for diflubenzuron to account for sensitive subgroups. Based on differences in methemoglobin reductase activity, a recovery mechanism for methemoglobinemia (Section 3.1.2), among different species, the factor of 10 for intraspecies variability appears adequate. The activity of this enzyme in humans appears to be about half of that in dogs (Calabrese 1991).

3.4.5. Connected Actions
The most sensitive effect for diflubenzuron, methemoglobinemia, is associated tebufenozide, another agent used for gypsy moth control. These two agents are likely to have an additive effect on methemoglobinemia but these agents are not used together. Thus, simultaneous exposures are unlikely. Exposure to other compounds in the environment that induce methemoglobinemia may

also lead to an additive effect. Individuals exposed to combustion smoke or carbon monoxide (that is, agents that do oxidative damage to blood) may be at increased risk of developing methemoglobinemia (Hoffman and Sauter 1989; Laney and Hoffman 1992). In addition, individuals exposed to high levels of nitrates, either in air or in water, will have increased levels of methemoglobin (Woebkenberg et al. 1981) and may be at increased risks of exposure to compounds such as diflubenzuron.

3.4.6. Cumulative Effects
This risk assessment is based on single applications at the maximum allowable rate, 70 g/ha. This is also the maximum rate that can be applied in a single season. This approach is used to estimate maximum daily exposure and daily absorbed dose. Because the dispersal rate for diflubenzuron in the environment is relatively fast, multiple applications at lower rates per application will result in risks that are less than those associated with a single application at the maximum approved rate. Given the narrow range of application rates compared with the variability and uncertainties in the exposure assessments, the risks of toxic effects associated with a single application at less than the maximum rate will be related directly to the application rate. Thus, an application at 35 g/ha will entail risks that are approximately one half of those expected at the maximum application rate.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION
4.1.1. Overview
The toxicity of diflubenzuron is well characterized in most groups of animals including mammals, birds, terrestrial invertebrates, fish and aquatic invertebrates. In general, diflubenzuron is much more toxic to some invertebrates, specifically arthropods, than vertebrates or other groups of invertebrates. This differential toxicity appears to involve fundamentally different mechanisms of action. Toxicity to sensitive invertebrate species is based on the inhibition of chitin synthesis. In the more tolerant vertebrate species, the mechanism of action appears to be a specific effect on the blood that inhibits oxygen transport.

The species most sensitive to diflubenzuron are arthropods, a large group of invertebrates including insects, crustaceans, spiders, mites, and centipedes. Most of these organisms use chitin, a polymer (repeating series of connected chemical subunits) of a glucose-based molecule, as a major component of their exoskeleton – i.e., outer body shell. Diflubenzuron is an effective insecticide because it inhibits the the formation of chitin. This effect disrupts the normal growth and development of insects and other arthropods. Both terrestrial and aquatic arthropods are affected but some substantial differences in sensitivity are apparent. In terrestrial organisms, the most sensitive species include lepidopteran and beetle larvae, grasshoppers and other herbivorous insects. More tolerant species include bees, flies, parasitic wasps, adult beetles, and sucking insects. In aquatic organisms, small crustaceans that consume algae and serve as a food source for fish (e.g., *Daphnia* species) appear to be the most sensitive to diflubenzuron while larger insect species such as backswimmers and scavenger beetles are much less sensitive. A wide range of other aquatic invertebrates, other crustaceans and small to medium sized aquatic insect larvae, appear to have intermediate sensitivities. Not all invertebrates utilize chitin and these invertebrates are much less sensitive to diflubenzuron than the arthropods. For terrestrial invertebrates, relatively tolerant species include earthworms and snails. For aquatic species, tolerant species include ostracods (an arthropod) and non-arthropods such as rotifers, bivalves (clams), aquatic worms, and snails.

As detailed in the human health risk assessment, the most sensitive effect in vertebrate species appears to involve damage to blood cells involved in the transport of oxygen. This effect has been demonstrated in mammals that are often employed in toxicity studies (e.g., rats and mice) as well as domestic animals and livestock. The effect has not been demonstrated in wildlife mammals, birds, or fish but it seems reasonable to assume that hemoglobin in all vertebrate species could be affected by exposure to diflubenzuron. Acute exposures to diflubenzuron are relatively non-toxic to mammals and birds. The U.S. EPA places diflubenzuron in low toxicity categories (III or IV) for mammals and considers diflubenzuron to be virtually non-toxic to birds in acute exposures and only slightly toxic to birds in subchronic exposures. This assessment is supported by a large number of field studies in which no direct toxic effects in mammals or birds have been reported. Effects, if any, on terrestrial vertebrates from the application of diflubenzuron are likely to be secondary to changes in food availability (i.e., reduced numbers of

insects) or changes in habitat (i.e., the protection of vegetation relative to untreated areas). Aquatic vertebrates also appear to be relatively tolerant to diflubenzuron and this compound is classified by U.S. EPA as practically non-toxic to fish. This classification appears to be appropriate and is supported by a relatively large number of longer term toxicity studies as well as field studies. Changes in fish populations have been noted in some studies but the changes appear to be secondary to changes in food supply. Although the data on amphibians are much more limited than the data in fish, a similar pattern is apparent – i.e., no direct toxic effects but changes in food consumption patterns secondary to effects on invertebrate species.

Data on plants and microorganisms are more limited than the data on invertebrates or vertebrates. Nonetheless, there does not appear to any basis for asserting that diflubenzuron will have a substantial effect on these organisms.

4.1.2. Toxicity to Terrestrial Organisms

4.1.2.1. Mammals – As summarized in Appendix 1 and discussed in the human health risk assessment (Section 3.1), there are a large number of toxicity studies on diflubenzuron in experimental mammals and these studies are relevant to the risk assessment for terrestrial mammals. Potential hazard to all wildlife mammals, however, may not be encompassed by the available data on experimental mammals – i.e., rats, mice, and dogs. As discussed in Section 3.1.3.1 and illustrated in Figure 3-1, some mammals such as sheep and pigs will metabolize diflubenzuron differently from rats. Specifically, metabolism in sheep, pigs, and perhaps other mammalian species, will result in cleavage of the ureido bridge with the formation of metabolites that are different from those seen in rats. There is little indication, however, that this difference in metabolism will lead to marked differences in toxicity. As summarized in Appendix 1, substantial differences in sensitivity among different species of mammals are not apparent. One possibly noteworthy difference, however, is a reduction in thyroid weight in sheep (Ross et al. 1977). As discussed in Section 3.1.8, the thyroid is an important organ in endocrine function. This effect, however, occurred in the absence of any signs of toxicity or changes in growth and may have been incidental.

The available field studies do not indicate any substantial impacts on mammalian wildlife from applications of diflubenzuron (Appendix 3a). As summarized in USDA (1995), applications of 60 to 280 g a.i./ha (0.85 to 4 oz a.i./ac) had no detectable adverse effects on the abundance of or reproduction in voles, field mice, and shrews (O'Connor and Moore 1975; Henderson et al. 1977). Small mammals increased in abundance on a plot receiving 280 g a.i./ha compared with a control plot (Henderson et al. 1977). The adverse effect that diflubenzuron might have on bot flies, a parasite of small as well as large mammals, was suggested as a possible explanation.

A more recent published field study by Seidel and Whitmore (1995) reports no effects on body measurements, weight, or fat content in populations of mice in areas treated with Dimilin 25 WP at a rate of rate of 140 g formulation/ha (35 g a.i./ha). Mice in the treated areas did consume less lepidopteran prey, secondary to the toxicity of diflubenzuron to lepidoptera, but total food consumption was not significantly different in treated and untreated plots.

4.1.2.2. Birds – A relatively large number of acute and subchronic toxicity studies are available in standard test species – i.e., mallard ducks and bobwhite quail – as well as other less commonly tested species – i.e., domestic hens and red-winged blackbirds (Appendix 4). Most of these studies were submitted to the U.S. EPA for the registration diflubenzuron (specified in Appendix 4 by MRID numbers) but some have been published in the open literature (e.g., Kubena 1981,1982, Kubena and Witzel 1980).

The acute toxicity of diflubenzuron to birds appears generally to be low and consistent with the gavage studies in rats in which gavage oral LD_{50} values are greater than 5000 mg/kg (Section 3.1 and Appendix 1). As summarized in Appendix 4, red-winged blackbirds appear to be somewhat more sensitive than mallard ducks – i.e., a gavage NOEL for red-winged blackbirds of 2500 mg/kg compared to a gavage NOEL for mallards of 5000 mg/kg. Nonetheless, diflubenzuron is classified a "virtually non-toxic" to both species as well as to bobwhite quail (U.S. EPA 1997a, p. 44). Based on the results of several standard reproduction studies, the the chronic dietary NOEC in birds is 500 ppm (U.S. EPA/OPP 1997a).

There is one atypical report of adverse reproductive effects in birds. Smalley (1976) reports that Dimilin (NOS), incorporated into the feed (dose not specified) of chicks (presumably chickens) for 13 weeks, resulted in an increased incidence of fat deposition in female chicks. The treated chicks weighed 6 ½ lbs, compared to normal weight of 3 lbs for controls (broilers) and males. In addition, Smalley (1976) reports a dose-related decrease in testosterone in treated males resulting in undeveloped combs, wattles, feathers, and voice. Very few experimental details are included in this study. Given the large number of other studies in birds in which no effects on reproduction were apparent, the report by Smalley (1976) appears to be an aberration.

The lack of direct effects on birds is supported by several field studies summarized in Appendix 3a. Some effects secondary to reduced lepidoptera prey may include increased foraging range (Cooper et al. 1990), relocation (Sample et al. 1993a,b) and lower body fat (Whitmore 1993).

4.1.2.3. Terrestrial Invertebrates – A large and relatively complex body of information is available on the toxicity of diflubenzuron to both target and non-target invertebrates. This information consists of both laboratory studies in which exposures are relatively well defined and controlled (Appendix 5) as well as field studies in which exposures are typically characterized as application rates (Appendix 3a).

A synopsis of the field studies in which exposures can be expressed in units of application rate (g/ha) are presented in Table 4-1. The first column in this table gives ranges of application rates spanning over an order of magnitude. The second and third columns provide species or groups of species in which no adverse effects (column 2) or adverse effects (column 3) were noted within the corresponding range of application rates. For each species or group the reference is given to a field study summarized in Appendix 3a. A similar summary table is not provided for the laboratory toxicity studies. As discussed further in the dose-response assessment

(Section 4.3.2.3), these studies were conducted using highly variable experimental designs and meaningful comparisons among the various toxicity assays summarized in Appendix 5 are difficult. Additional details of the comparisons among the various field studies are also provided in the dose-response assessment (see discussion of Table 4-5 in Section 4.3.2.3).

The insecticidal action of diflubenzuron is based on the inhibition of chitin synthesis. Chitin is a polymer (repeating series of connected chemical subunits) of a glucose-based molecule and comprises a substantial proportion of the exoskeleton (outer-shell) of insects. Consequently, the inhibition of chitin synthesis disrupts the growth and development of insects. Chitin is also contained in other arthropods (i.e., crustaceans, spiders, and centipedes) as well as some fungi. Thus, the mode of action of diflubenzuron as a insecticide to target species is also relevant to effects on non-target insects as well as other arthropods (Cardona 1999; Cunningham 1986; Eisler 1992; Fisher and Hall 1992; Hobson 2001; Lengen, 1999; Wilson 1997; Wilcox and Coffey 1978). Diflubenzuron also exerts ovicidal effects in several species (Ables et al. 1977; Büchi and Jossi, 1979; Kumar et al. 1994;) and has been shown to inhibit egg production in some species (Rumpf et al. 1998; Medina et al. 2002; Medina et al. 2003).

While the mechanism of action of diflubenzuron is not specific to target insects, there is ample data indicating substantial differences in sensitivity among various groups of terrestrial invertebrates. Invertebrates without exoskeletons, such as earthworms and snails, do not utilize chitin and diflubenzuron is relatively non-toxic to these species (Berends and Thus 1992; Berends et al. 1992). Even among different groups of arthropods, however, differences in sensitivity to diflubenzuron seem apparent. Species that are most sensitive to diflubenzuron include lepidopteran and beetle larvae, grasshoppers and other chewing herbivorous insects (Berry et al. 1993; Butler 1993; Butler et al. 1997; Elliott and Iyer 1982; Jepson and Yemane 1991; Jepson and Martinat et al. 1998, 1993; Kumar et al. 1994; McWhorter and Shapard 1971; Sample et al.1993b; Sinha et al. 1990; Redfern et al. 1980; Yemane 1991). Other species are relatively tolerant to diflubenzuron. These include flies, wasps that are parasites on insect eggs, adult beetles, and sucking insects (Ables et al. 1975; Broadbent and Pree, 1984a; Brown and Respicio, 1981; Bull and Coleman, 1985; De Clercq et al. 1995b; Deakle and Bradley 1981; Delbeke et al. 1997; Gordon and Cornect, 1986; Keever et al. 1977; Martinat et al., 1988; Webb et al. 1989; Zacarias et al. 1998; Zungoli et al. 1983).

The honey bee is a standard test species used by U.S. EPA to classify the toxicity of pesticides to non-target invertebrates. Based on early acute oral and contact toxicity studies in honey bees with LD_{50} values of >30 µg/bee and >114.8 µg/bee (Atkins et al. 1974; Stevenson 1978), the U.S. EPA (1997a) has classified diflubenzuron as *"practically non-toxic to honey bees"* (U.S. EPA 1997a, p. 81). As discussed further in the dose-response assessment (Section 4.3.2.3), several other laboratory toxicity studies also indicate that diflubenzuron is not highly toxic to bees (Chandel and Gupta 1992; Elliott and Iyer, 1982; Gijswijt, 1978; Kuijpers, 1989; Nation et al. 1986; Yu et al. 1984) and this is supported for several field studies conducted at application rates comparable to or substantially higher than those used to control the gypsy moth (Buckner et al. 1975; Emmett and Archer 1980; Matthenius, 1975; Schroeder 1978a; Schroeder 1980). In

addition, no detectable amounts of diflubenzuron were found in honey bees in areas treated with diflubenzuron (Cochran and Poling 1995). Some studies have noted adverse effects in bees. As summarized in Appendix 5, Stoner and Wilson (1982) and (Thompson and Wilkins 2003) noted transient decreases in brood production at relatively high concentrations (10 ppm) in longer term exposures. At 1 ppm or less, however, no effects were noted. Barrows (1995) noted a decrease in the mean number of pollinating insects in watersheds during a year in which diflubenzuron was applied but not in the following year.

In addition to the acute toxic effects of diflubenzuron, mediated primarily through inhibition of chitin, adverse reproductive effects have been reported in several different orders of insects including moths (Beevi and Dale 1984; Tembhare and Shinde 1998), beetles (Büchi and Jossi 1979; Khebbeb et al. 1997; Mani et al. 1997; Soltani and Soltani-Mazouni 1994a,b,1995a,b,1997), grasshoppers (Mathur 1998), lacewings (Medina et al. 2002; Medina et al. 2003; Rumpf et al. 1998), and true bugs – i.e., Order Hemiptera including the suborder Heteroptera (Redfern et al. 1980; Sindhu and Muraleedharan 1997).

In Lepidoptera, reproductive effects were reported by Beevi and Dale (1984), who noted a high incidence of sterility in the rice swarming caterpillar (*Spodoptera mauritania*) after exposures to relatively high concentrations of Dimilin – 10 ppm and higher. The mechanism of this reproductive effect is unclear but may involve the endocrine system – i.e., hormone release by neurosecretory cells. This has been noted in larvae of the fruit-sucking moth, *Othreis materna* (Tembhare and Shinde 1998) and in the cotton bug (*Dysdercus cingzrlattis*) (Sindhu and Muraleedharan 1997). In some other species of Lepidoptera – i.e., tufted apple bud moth – pupae are sensitive to diflubenzuron but no effects are apparent on reproduction (Biddinger and Hull 1999).

In beetles (Coleoptera), effects on larvae, eggs, and reproductive performance have been noted (Büchi and Jossi1979; Mani et al. 1997). In the mealworm, diflubenzuron impacts lipid metabolism in fat bodies and ovaries (Khebbeb et al. 1997). A series of studies in this species (Soltani and Soltani-Mazouni 1997; Soltani-Mazouni and Soltani1994a,b, 1995b) suggest that the decreased fecundity observed in this and other insect species may be associated with the effect of diflubenzuron on oogenesis, possibly due to changes vitellogenic precursors, the production of ecdysteroid by follicle cells, and/or the inhibition of ovarian DNA synthesis. Direct damage to ovary tissue has also been observed in one species of Orthoptera, a grasshopper, but the mechanism of action in this species has not been studied (Mathur 1998).

Reproductive effects in lacewings (Neuroptera) have been noted by Rumpf et al. (1998) and Medina et al. (2002, 2003). As detailed in Appendix 5, contact exposures to diflubenzuron at 0.07 µg/cm^2 resulted in a substantial decrease in egg production and complete infertility in 13% of the exposed animals. No effects on egg production or hatching in this species have been observed after direct topical applications at doses as low as 0.5 ng/insect. At a substantially higher dose, 75 ng/insect, egg hatching was reduced by 32%. (Medina et al. 2002, 2003).

4.1.2.4. Terrestrial Plants (Macrophytes) – As noted in U.S. EPA/OPP (1997a), no terrestrial plant toxicity studies had been submitted to the U.S. EPA at the time of the reregistration of diflubenzuron. In the literature search conducted for the current risk assessment, no bioassays for herbicidal activity of diflubenzuron were encountered in either the published literature or in the more recent U.S. EPA/OPP files.

There are a large number of terrestrial field studies regarding the efficacy of diflubenzuron applied to terrestrial vegetation for the control of various insect pests including the gypsy moth (Appendix 3a). If diflubenzuron were toxic to terrestrial plants at application rates that are used in the field, it is plausible that adverse effects would have been reported in this literature. No such reports were encountered. Thus, there is no basis for asserting that diflubenzuron will cause adverse effects in terrestrial plants and such effects will not be considered quantitatively in this risk assessment.

4.1.2.5. Terrestrial Microorganisms – As discussed in Section 3.2 and summarized in Appendix 2 (Environmental Fate) and Appendix 3a (Terrestrial Field Studies), diflubenzuron is readily degraded by terrestrial microorganisms. The degradation of diflubenzuron by soil microorganisms suggests that this compound is not toxic to soil microorganisms and this presumption may account for the relatively few studies on microbial toxicity. Fungi, however, do contain chitin in cell walls and thus could be a potential target. Booth (1978) found no inhibition of fungal growth in several species of fungi (*Aspergillus*, *Fusarium*, *Rhizopus*, *Trichoderma*) at concentrations of up to 100 ppm in growth media – i.e. mg diflubenzuron per kg of soil. Some growth inhibition, however, was noted in a species of *Pythium* at a concentration of 50 ppm. Inhibition of *Rhizoctonia solani*, another terrestrial fungus, has been noted at 300 ppm (Townshend et al. 1983).

The lack of microbial toxicity was also specifically noted in one field study in which no effects on soil or litter populations of bacteria, actynomycetes or fungi were noted after applications of diflubenzuron at a rate of 67.26 g/ha (Kurczewski et al. 1975; Wang 1975), field and laboratory studies on molds and leaf litter or soil bacteria (Landolt and Stephenson 1995), and studies on mycorrhizal or debris decomposing fungi (Iskra et al. 1995; Gundrum et al. 1995).

One study has noted minor and transient changes in microbial activity. Sexstone (1995) conducted a laboratory study in which soil cores were treated at $4.418\mu g/44.2$ cm^2, roughly equivalent to an application rate of 10 g/ha [$4.418\mu g/44.2$ cm$^2 \times 10,000$ cm^2/m$^2 \times 10,000$ m^2/ha = 9,995,475 μg/ha $\approx$ 10 g/ha]. Only transient and sporadic decreases were noted in microbial biomass [Figure 14-1 in Sexton 1995]. These changes in microbial activity were apparent up to day 35 after treatment but there were no changes by 64 days after treatment. Changes in respiration [Figure 14-2 in Sexton 1995] and nitrification [Figures 14-3 to 14-6 in Sexton 1995] and appear to insubstantial. While some of the differences were statistically significant at some time points, Sexstone (1995) characterizes the effects a "minor" and this assessment appears reasonable.

4.1.3. Aquatic Organisms.

4.1.3.1. Fish – The toxicity of diflubenzuron to fish is well characterized in terms of both acute and chronic toxicity and one mesocosm study is available (Appendix 6). In addition, several of the aquatic field studies (Appendix 3b) involve observations on fish populations. Diflubenzuron has a low order of acute toxicity to fish, with 96-hour LC_{50} values in the range of over 25 mg/L(the value for yellow perch reported by Johnson and Finley 1980) to over 500 mg/L (the value for fathead minnow reported by Reiner and Parke 1975). In addition to data on technical grade diflubenzuron, some studies have also been conducted on Dimilin 25W (Julin and Sanders 1978 with additional studies summarized in U.S. EPA 1997a) and these studies indicate that the toxicity of Dimilin 25W is not greater than the toxicity of technical grade diflubenzuron. No studies have been encountered on the acute toxicity of Dimilin 4L to fish. Based on the available information, the U.S. EPA (1997a, p. 47) has classified diflubenzuron as *"practically non-toxic"* to fish in terms of risks from acute exposures.

Diflubenzuron also appears to be relatively non-toxic to fish in longer term exposures. One standard assay for longer term toxicity in fish involves exposing fish eggs to a compound and maintaining the exposure through to the fry stage. In this type of assay, concentrations up to 45 ppb has no effect on egg or fry of steelhead trout, fathead minnows, or guppies (Hansen and Garton 1982a). In addition, no effects were seen in longer-term studies at concentrations up to 100 ppb (Cannon and Krize 1976) or in 2-generation reproduction studies at concentrations of up to 50 ppb (Livingston and Koenig 1977).

As discussed in Section 4.1.3.2, diflubenzuron is much more toxic to invertebrates than to fish and indirect effects on fish are plausible based on a decrease in invertebrate populations. Such effects have been demonstrated in mesocosm studies (Moffett and Tanner 1995; Tanner and Moffett 1995) in which concentrations as low as 2.5 ppb resulted in decreased growth of fish in littoral enclosures – i.e., populations of fish placed and monitored in enclosures along the shore of a body of water. The reduced growth observed in these studies was attributed to a reduction in macroinvertebrates that serve as a food source for the fish.

It is unclear, however, that secondary effects on fish growth or populations will be observed in the field. None of the field studies summarized in Appendix 3b note any adverse effects on fish in applications comparable to or greater than those used in the control of the gypsy moth. For example, Farlow et al. (1978) conducted a relatively large field study in a marsh area treated with six applications of diflubenzuron at 28 g a.i./ha – i.e., a cumulative application of 168 g/ha. While substantial shifts were noted in various invertebrates (Appendix 3a and Section 4.1.3.2), populations of mosquito fish (*Gambusia affinis*) and American flag fish (*Jordanella floridae*) increased. Similarly, no effects on the growth of fish were noted in ponds directly treated with diflubenzuron at a concentration of 5 ppb (Apperson et al. 1977, 1978) or 13 ppb (Colwell and Schaefer 1980). The study by Colwell and Schaefer (1980) did note a shift in diet of fish (secondary to changes in food availability) but no effect on growth rates or general condition of the fish.

4.1.3.2. Amphibians – Amphibians are not standard test organisms for toxicity studies and no standard bioassays on amphibians have been encountered in the open literature or U.S. EPA/OPP files. Two field studies (Pauley 1995a,b), however, are available on salamanders. Both of these studies were conducted as part of a large study on the effects of spraying diflubenzuron in the northeast for control of the gypsy moth (Reardon 1995a). In this study, two watersheds were treated with Dimilin 4L in 1992 at a rate of 80g/ha (0.03 lb/acre) (Reardon 1995b). Pauley (1995a,b) conducted field studies to assess effects on both aquatic (Pauley 1995a) and terrestrial salamanders (Pauley 1995b). While all salamanders are amphibians, some species spend most of their time on land while others spend most of their time in water. In aquatic salamanders, diflubenzuron treatment was associated with a shift in dietary consumption to more hard-bodied prey secondary to a reduction in the availability of soft-bodied prey. This is similar to the pattern with fish as noted above. No effects in salamanders, however, were noted based on body size or population (Pauley 1995). In terrestrial salamanders, similar results were observed with no change in body size or body fat associated with treatment but a shift was seen in food consumption to hard-bodied prey (Pauley 1995b).

4.1.3.3. Aquatic Invertebrates – As summarized in Appendix 7, there is a very large and diverse body of literature indicating that diflubenzuron is highly toxic to many aquatic invertebrates. Because diflubenzuron inhibits the synthesis of chitin, crustaceans (arthropods which rely on chitin synthesis for the formation of the exoskeleton) are the aquatic invertebrates that are most sensitive to diflubenzuron.

One of the most common crustacean species used in freshwater invertebrate toxicity studies is *Daphnia magna*, a member of Daphnidae in the order Cladocera. These and other zooplankton feed on aquatic algae and are a source of food for fish. Many bioassays, both acute and chronic, have been conducted on *Daphnia magna* (Hansen and Garton 1982a; Kuijpers 1988; Majori et al. 1984; Surprenant 1988) as well as a related species, *Ceriodaphnia dubia* (Hall 1986). As detailed further in the dose-response assessment, these organisms are among the most sensitive to diflubenzuron, with acute LC_{50} values of about 2 µg/L (Hall 1986; Hansen and Garton 1982a). Several other crustacean species appear to be about as sensitive or only somewhat less sensitive to diflubenzuron as daphnids (Appendix 7).

Broad generalizations are somewhat difficult to make, however, because of the diversity of the studies that have been conducted. Nonetheless, large insects appear to be much more tolerant to diflubenzuron than crustaceans, with acute LC_{50} values on the order of 2123 µg/L for backswimmers (Lahr et al. 2001) and an NOEC of 250 µg/L for scavenger beetles (Miura and Takahashi 1974).

Organisms that do not rely on chitin for an exoskeleton are much less sensitive to diflubenzuron. In the microcosm study by Corry et al. (1995) concentrations of diflubenzuron that caused adverse effects in cladocerans caused no adverse effects in rotifers – an aquatic invertebrate that lacks an exoskeleton. Similar tolerance in rotifers have been observed in littoral enclosure studies at diflubenzuron concentrations of up to 30 µg/L (Liber and O'Halloran 1995). At about

the same concentration, 30 µg/L, two species of snails and aquatic worms were not affected by exposures to diflubenzuron (Hansen and Garton 1982a,b). One common genus of snail, *Physa*, had a reported LC_{50} value of greater than 125 mg/L – i.e., 125,000 µg/L. Ostracods (small bivalve crustaceans) were not affected by diflubenzuron at concentrations up to 2.5 µg/L (Liber and O'Halloran 1995) and much larger Quahog clams (*Mercinaria mercinaria*) were unaffected at concentrations up to 320 µg/L (Surprenant 1989).

As with fish, no data have been located on the toxicity of Dimilin 4L. Lahr (2000, 2001) used a "solvent based" formulation of diflubenzuron but did not specify the formulation as Dimilin 4L. The 48-hour EC_{50} of 0.74 µg/L (0.60-0.88 µg/L) of the solvent based formulation in fairy shrimp, *Streptocephalus sudanicus* reported by Lahr (2001) is comparable to EC_{50} value of 0.65 µg/L for technical grade diflubenzuron reported in grass shrimp, *Palaemonetes pugio* (Tourat and Rao 1987). Toxicity studies are available on Dimilin 25W and, as with fish, the toxicity of Dimilin 25W appears to be the same as technical grade diflubenzuron when exposures are expressed in units of active ingredient (Wilson and Costlow 1986). Thus, there does not appear to be a basis for asserting that the formulated products containing diflubenzuron are more hazardous than diflubenzuron itself.

The available field studies on the effects of diflubenzuron on aquatic invertebrates reenforce the standard toxicity studies, indicating that diflubenzuron will impact invertebrate populations. Several of these studies, however, were conducted at application rates substantially higher than those used to control the gypsy moth. As noted in the program description (Section 2), the maximum application rate that will be used in USDA programs is about 70 g/ha. Many of the studies in which severe adverse effects were observed in aquatic invertebrate populations involved multiple applications at rates between about 110 g/ha and 560 g/ha (e.g., Ali and Mulla 1978a,b; Ali et al. 1988; McAlonan 1975). Similarly, other field studies involve direct applications to open water, a treatment method that is not part of USDA program activities, and which resulted in water concentrations that are in the range of 10 ppb (e.g., Apperson et al. 1977; Boyle et al. 1996; Colwell and Schaefer 1980; Lahr et al. 2000; Sundaram et al. 1991). As discussed further in Section 4.2, concentrations of 10 ppb or greater are in the range of peak concentrations that are likely to be encountered in USDA programs. Concentrations in the range of 10 ppb, however, are substantially higher than average concentrations of diflubenzuron in water that are likely to be encountered in USDA programs.

Those field studies that used lower application rates more typical of USDA programs (e.g., Farlow 1976; Griffith et al. 1996; Griffith et al. 2000; Hurd et al. 1996; Jones and Kochenderfer 1987; Reardon 1995a) have noted some effects on freshwater invertebrates, particularly smaller crustaceans, but the effects were much less severe than those seen in the higher application rate studies. This is discussed further in Section 4.4 (Risk Characterization).

4.1.3.4. Aquatic Plants – Data on the toxicity of diflubenzuron to aquatic plants is summarized in Appendix 8. Most studies report no direct toxic effects of diflubenzuron on aquatic plants (algae or macrophytes) at concentrations of 100 µg/L or higher (Booth and Ferrell 1977;

Thompson and Swigert 1993a,b,c) and no indirect effects on aquatic macrophytes (Moffett 1995). A decrease in periphyton in littoral enclosures, however, was noted by Moffett (1995) at 7.0, or 30 µg/L but not at 0.7 or 2.5 µg/L. This effect was attributed not to a direct toxic effect on the periphyton but to the loss of grazers (e.g., cladocera) that may have induced premature senescence in periphyton secondary to a decrement in water quality.

4.1.3.5. Aquatic Microorganisms – There is very little information suggesting that diflubenzuron will adversely affect aquatic microorganisms. No marked differences in numbers of fungal taxa in treated and untreated watersheds were noted by Dubey (1995) in a survey of watersheds treated with diflubenzuron for the control of the gypsy moth. In an aquatic mesocosm, Kreutzweiser et al. (2001) did note a slight but significant effect of diflubenzuron (50 µg/L and 50,000 µg/L) on microbial decomposition and respiration. Changes at 50 µg/L, however, were only marginally significant and variable over the 21-day period.

In the Kreutzweiser et al. (2001) study, Dimilin 4L was used. This is the only laboratory study involving Dimilin 4L. Because no corresponding studies are available on Dimilin 25W or technical grade diflubenzuron, inferences concerning the potential effect of the petroleum solvent in Dimilin 4L cannot be made.

4.2. EXPOSURE ASSESSMENT
4.2.1. Overview
As in the human health risk assessment (Section 3.2), exposures are estimated for both diflubenzuron and 4-chloroaniline. A full set of exposure assessments are developed for diflubenzuron but only a subset of exposure assessments are developed for 4-chloroaniline. This approach is taken, again as in the human health risk assessment, because 4-chloroaniline is assessed as an environmental metabolite of diflubenzuron. Thus, immediately after application, the amount of 4-chloroaniline as an environmental metabolite will be negligible. Consequently, the direct spray scenarios as well as the consumption of insects and the consumption of small mammals after a direct spray are not included for 4-chloroaniline. Also as in the human health risk assessment, all standard chronic exposure scenarios are included for 4-chloroaniline. Details of the exposure assessments for diflubenzuron and 4-chloroaniline are given in the two sets of worksheets that accompany this risk assessment: Supplement 1for diflubenzuron and Supplement 2 for 4-chloroaniline. All exposure assessments are based on the maximum application rate of 70 g/ha.

Terrestrial animals might be exposed to any applied pesticide from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation. For diflubenzuron, the highest acute exposures for small terrestrial vertebrates will occur after a direct spray and could reach up to about 10 mg/kg at an application rate of 70 g/ha. Exposures anticipated from the consumption of contaminated vegetation by terrestrial animals range from central estimates of about 0.08 mg/kg for a small mammal to 2 mg/kg for a large bird with upper ranges of about 0.2 mg/kg for a small mammal and 5 mg/kg for a large bird. The consumption of contaminated water leads to much lower levels of exposure. A similar pattern is seen for chronic exposures. Estimated longer-term daily doses for a small mammal from the consumption of contaminated vegetation at the application site are in the range of about 0.001 mg/kg to 0.005 mg/kg. Large birds feeding on contaminated vegetation at the application site could be exposed to much higher concentrations, ranging from about 0.08 mg/kg/day to 0.7 mg/kg/day. The upper ranges of exposure from contaminated vegetation far exceed doses that are anticipated from the consumption of contaminated water, which range from about 0.0000001 mg/kg/day to 0.00001 mg/kg/day for a small mammal.

Exposures of terrestrial organisms to 4-chloroaniline tend to be much lower than those for diflubenzuron. The highest acute exposure is about 0.2 mg/kg, the approximate dose for the consumption of contaminated water by a small mammal and the consumption of contaminated fish by a predatory bird. The highest longer term exposure is 0.0002 mg/kg/day, the dose associated with the consumption of contaminated vegetation by a large bird.

Exposures to aquatic organisms are based on the same information used to assess the exposures of terrestrial species from contaminated water. At the maximum application rate of 70 g/ha, the upper range of the expected peak concentration of diflubenzuron in surface water is taken as 16 µg/L. The lower range of the concentration in ambient water is estimated at 0.01 µg/L. The central estimate of concentration of diflubenzuron in surface water is taken as 0.4 µg/L.

4.2.2. Terrestrial Animals
Terrestrial animals might be exposed to any applied insecticide from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation.

In this exposure assessment, estimates of oral exposure are expressed in the same units as the available toxicity data. As in the human health risk assessment, these units are usually expressed as mg of agent per kg of body weight and abbreviated as mg/kg for terrestrial animals. One exception in this risk assessment involves terrestrial invertebrates. As detailed in the dose-response assessment (Section 4.3), toxicity data in units of mg/kg bw are available for some terrestrial invertebrates and these data are used in a manner similar to that for terrestrial vertebrates. For other species, however, standard toxicity studies report units that are not directly useful in a quantitative risk assessments – e.g., contact toxicity based on petri dish exposures. As an alternative, some dose response assessments are based on field studies in which the dose metameter is simply the application rate in units of mass per area such as g a.i./ha.

For dermal exposures to terrestrial animals, the units of measure usually are expressed in mg of agent per cm^2 of surface area of the organism and abbreviated as mg/cm^2. In estimating dose, however, a distinction is made between the exposure dose and the absorbed dose. The *exposure dose* is the amount of material on the organism (i.e., the product of the residue level in mg/cm^2 and the amount of surface area exposed), which can be expressed either as mg/organism or mg/kg body weight. The *absorbed dose* is the proportion of the exposure dose that is actually taken in or absorbed by the animal.

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

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

insects by a small mammal (Worksheet F14a) and the consumption by a large mammalian carnivore of small mammals contaminated by direct spray (Worksheet F16a). Exposure scenarios for birds involve the consumption of contaminated insects by a small bird (Worksheet F14b), the consumption of contaminated fish by a predatory bird (Worksheets F08 and F09), the consumption by a predatory bird of small mammals contaminated by direct spray, and the consumption of contaminated grasses by a large bird (F12, F13a, and F13b).

While a very large number of other exposure scenarios could be generated, the specific exposure scenarios developed in this section are designed as conservative screening scenarios that may serve as guides for more detailed site-specific assessments by identifying the groups of organisms and routes of exposure that are of greatest concern.

4.2.2.1. Direct Spray – In the broadcast application of any insecticide, wildlife species may be sprayed directly. This scenario is similar to the accidental exposure scenarios for the general public discussed in Section 3.2.3.2. In a scenario involving exposure to direct spray, the amount absorbed depends on the application rate, the surface area of the organism, and the rate of absorption.

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

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

Because of the relationship of body size to surface area, very small organisms, like bees and other terrestrial invertebrates, might be exposed to much greater amounts of a pesticide per unit body weight compared with small mammals. Consequently, a third exposure assessment is developed using a body weight of 0.093 g for the honey bee (USDA/APHIS 1993) and the

equation above for body surface area proposed by Boxenbaum and D'Souza (1990). Because there is no information regarding the dermal absorption rate of diflubenzuron by bees or other invertebrates, this exposure scenario, detailed in Worksheet F02b, also assumes complete absorption over the first day of exposure. As noted above, exposures for other terrestrial invertebrates are based on field studies in which application rate is the most relevant expression of exposure. This is discussed further in Section 3.3 (Dose-Response Assessment) and Section 3.4 (Risk Characterization).

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

4.2.2.2. Indirect Contact – As in the human health risk assessment (see Section 3.2.3.3), the only approach for estimating the potential significance of indirect dermal contact is to assume a relationship between the application rate and dislodgeable foliar residue. Unlike the human health risk assessment in which transfer rates for humans are available, there are no transfer rates available for wildlife species. As discussed in Durkin et al. (1995), the transfer rates for humans are based on brief (e.g., 0.5 to 1-hour) exposures that measure the transfer from contaminated soil to uncontaminated skin. Wildlife, compared with humans, are likely to spend longer periods of time in contact with contaminated vegetation. It is reasonable to assume that for prolonged exposures a steady state may be reached between levels on the skin, rates of absorption, and levels on contaminated vegetation, although there are no data regarding the kinetics of such a process. The bioconcentration data on diflubenzuron indicates that this compound will accumulate in the tissue of the fish. Thus, it is plausible that absorbed dose resulting from contact with contaminated vegetation will be as great as those associated with comparable direct spray scenarios.

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

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

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

A large herbivorous mammal is included because empirical relationships of concentrations of pesticides in vegetation, discussed below, indicate that grasses may have substantially higher pesticide residues than other types of vegetation such as forage crops or fruits (Worksheet B21). Grasses are an important part of the diet for some large herbivores, but most small mammals do not consume grasses as a substantial proportion of their diet. Thus, even though using residues from grass to model exposure for a small mammal is the most conservative approach, it is not generally applicable to the assessment of potential adverse effects. Hence, in the exposure scenarios for large mammals, the consumption of contaminated range grass is modeled for a 70 kg herbivore, such as a deer. Caloric requirements for herbivores and the caloric content of vegetation are used to estimate food consumption based on data from U.S. EPA/ORD (1993). Details of these exposure scenarios are given in Worksheet F10 for acute exposures as well as Worksheets F11a and F11b for longer-term exposures.

For the acute exposures, the assumption is made that the vegetation is sprayed directly – i.e., the animal grazes on site – and that100% of the animals diet is contaminated. While appropriately conservative for acute exposures, neither of these assumptions are plausible for longer-term exposures. Thus, for the longer-term exposure scenarios for the large mammal, two sub-scenarios are given. The first is an on-site scenario that assumes that a 70 kg herbivore consumes short grass for a 90 day period after application of the chemical. In the worksheets, the contaminated vegetation is assumed to account for 30% of the diet with a range of 10% to 100% of the diet. These are essentially arbitrary assumptions reflecting grazing time at the application site by the animal. Because the animal is assumed to be feeding at the application site, drift is set to unity - i.e., direct spray. This scenario is detailed in Worksheet 11a. The second sub-scenario is similar except the assumption is made that the animal is grazing at distances of 25 to 100 feet from the application site (lowering risk) but that the animal consumes 100% of the diet from the contaminated area (increasing risk). For this scenario, detailed in Worksheet F12b, AgDRIFT is used to estimate deposition on the off-site vegetation. Drift estimates from AgDrift are summarized in Worksheet B24 and this model is discussed further in Section 4.2.3.2.

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

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

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types of vegetation. As summarized in Worksheet B21, these residue rates are based on estimated residue rates from Fletcher et al. (1994).

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

A similar set of scenarios is provided for the consumption of small mammals by either a predatory mammal (Worksheet F16a) or a predatory bird (Worksheet F16b). Each of these scenarios assumes that the small mammal is directly sprayed at the specified application rate and the concentration of the compound in the small mammal is taken from the worksheet for direct spray of a small mammal under the assumption of 100% absorption (Worksheet F02a).

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

4.2.2.4. Ingestion of Contaminated Water – Estimated concentrations of diflubenzuron in water are identical to those used in the human health risk assessment (Worksheet A04). The only major differences involve the weight of the animal and the amount of water consumed. There are well-established relationships between body weight and water consumption across a wide range of mammalian species (e.g., U.S. EPA 1989). Mice, weighing about 0.02 kg, consume approximately 0.005 L of water/day (i.e., 0.25 L/kg body weight/day). These values are used in the exposure assessment for the small (20 g) mammal. Unlike the human health risk assessment, estimates of the variability of water consumption are not available. Thus, for the acute scenario, the only factors affecting the variability of the ingested dose estimates include the field dilution rates (i.e., the concentration of the chemical in the solution that is spilled) and the amount of solution that is spilled. As in the acute exposure scenario for the human health risk assessment, the amount of the spilled solution is taken as 200 gallons. In the exposure scenario involving contaminated ponds or streams due to contamination by runoff or percolation, the factors that affect the variability are the water contamination rate, (see Section 3.2.3.4.2) and the application rate. Details regarding these calculations are summarized in Worksheets F06 and Worksheet F07.

4.2.3. Terrestrial Plants

Terrestrial plants will certainly be exposed to diflubenzuron. A large number of different exposure assessments could be made for terrestrial plants – i.e., direct spray, spray drift, runoff, wind erosion and the use of contaminated irrigation water. Such exposure assessments are typically conducted for herbicides. For diflubenzuron, however, the development of such exposure assessments would serve no purpose. As discussed in Section 4.1.2.4 (Hazard Identification for Terrestrial Plants), there is no basis for asserting that diflubenzuron will cause adverse effects in terrestrial plants. Thus, no formal exposure assessment is conducted for terrestrial plants.

4.2.4. Soil Organisms

For both soil microorganisms and soil invertebrates, the toxicity data are typically expressed in units of soil concentration – i.e., mg agent/kg soil which is equivalent to parts per million (ppm) concentrations in soil. The GLEAMS modeling, discussed in Section 3.2.3.4, provides estimates of concentration in soil as well as estimates of off-site movement (runoff, sediment, and percolation). Based on the GLEAMS modeling, concentrations in clay, loam, and sand over a wide range of rainfall rates are summarized in Table 4-2. As indicated in this table, peak soil concentrations at an application rate of 70 g/ha are in a relatively narrow range: about 0.003 to 0.009 mg/kg (ppm) over all soil types and rainfall rates. Longer term concentrations in soil are all low and are on the order of 0.00005 to 0.0005 mg/kg – i.e., 0.05 ppb to 0.5 ppb. Modeled concentrations of 4-chloroaniline in soil are summarized in Table 4-3. As would be expected of any environmental metabolite, peak concentrations are lower than those of the parent compound. For 4-chloroaniline these range from about 0.0007 to 0.003 mg/kg, about a factor of three lower than the corresponding concentrations of diflubenzuron.

4.2.5. Aquatic Organisms

The potential for effects on aquatic species are based on estimated concentrations of diflubenzuron and 4-chloroaniline in water that are identical to those used in the human health risk assessment. As summarized in Table 3-8, the peak estimated concentration of diflubenzuron in ambient water is 0.4 (0.01 to 16) µg/L at an application rate of 70 g/ha. For longer-term exposures, the corresponding longer term concentrations in ambient water are estimated at 0.02 (0.001 to 0.1) µg/L. The corresponding estimates for 4-chloroaniline are summarized in Table 3-9: 0.5 (0.00003 to 2) µg/L for acute exposures and 0.05 (0.0002 to 0.2) µg/L for longer term exposures.

4.3. DOSE-RESPONSE ASSESSMENT
4.3.1. Overview
As in the human health risk assessment, toxicity values are derived for both diflubenzuron and 4-chloroaniline. Several of the toxicity values used in the ecological risk assessment for diflubenzuron are summarized in Table 4-4. For two groups of organisms, terrestrial arthropods and aquatic invertebrates, detailed dose-response assessments can be made for several different subgroups. These toxicity values are summarized in Table 4-5 for terrestrial arthropods and Table 4-6 for aquatic invertebrates. The values for 4-chloroaniline are summarized in Table 4-7.

Diflubenzuron is relatively non-toxic to mammals and birds. For mammals, the toxicity values used in the ecological risk assessment are identical to those used in the human health risk assessments: an acute NOAEL of 1118 mg/kg and a chronic NOAEL of 2 mg/kg/day. A similar approach is taken for 4-chloroaniline for which an acute NOAEL of 8 mg/kg is used based on a subchronic study and a chronic NOAEL is estimated at 1.25 mg/kg/day based on the chronic LOAEL of 12.5 mg/kg/day. For birds, the acute NOAEL for diflubenzuron is taken as 2500 mg/kg from an acute gavage study and the longer term NOAEL is taken as 110 mg/kg/day from a reproduction study. No data are available on toxicity of 4-chloroaniline in birds and the available toxicity values for mammals are used as a surrogate.

For terrestrial invertebrates two general types of data could be used to assess dose-response relationships: laboratory toxicity studies and field studies. Field studies are used in the current risk assessment because the standard toxicity studies are extremely diverse and many are not directly applicable to a risk assessment. Despite the difficulty and uncertainty in interpreting some of the field studies, the relatively large number of field studies on diflubenzuron appear to present a reasonably coherent pattern that is at least qualitatively consistent with the available toxicity data and probably a more realistic basis on which to assess risk to nontarget species. The most sensitive species appear to be grasshoppers which may be adversely affected at an application rate of 22 g/ha. Somewhat high application rates – in the range of 30 to 35 g/ha – will adversely affect macrolepidoptera and some beneficial parasitic wasps. At the maximum application rate considered in this risk assessment – i.e., 70 g/ha – some herbivorous insects are likely to be affected. No adverse effects in several other groups of insects are expected at this or much higher application rates. Honeybees are among the most tolerant species and are not likely to be adversely affected at application rates of up to 400 g/ha.

Invertebrates that do not utilize chitin are also relatively insensitive to diflubenzuron. The NOEC for a species of earthworm (*Eisenia fetida*) is 780 mg/kg soil and is used to represent tolerant species of soil invertebrates. Very little information is available on the toxicity of 4-chloroaniline to terrestrial invertebrates. As with diflubenzuron, the earthworm appears to be relatively tolerant to 4-chloroaniline with a reported LC_{50} value of 540 mg/kg dry soil. The toxicity of both diflubenzuron and 4-chloroaniline to soil microorganisms is also relatively low.

Toxicity values for aquatic species follow a pattern similar to that for terrestrial species: arthropods appear to be much more sensitive than fish or non-arthropod invertebrates. For

diflubenzuron, LC_{50} values of 25 mg/L to 500 mg/L are used to characterize risks for sensitive and tolerant species of fish, respectively. 4-Chloroaniline appears to be more toxic to fish and an LC_{50} value of 2.4 mg/L is used to characterize risks of peak exposures and 0.2 mg/L is used to characterize risks of longer term exposures.

Substantial variability in the response of different groups of aquatic invertebrates to diflubenzuron is apparent. Very small arthropods appear to be among the most sensitive species – with acute NOEC values in the range of 0.3 to about 1 ppb (µg/L) and chronic NOEC values in the range of 0.04 to 0.25 ppb. Based on acute NOEC values, larger arthropods, including crabs and larger insects, appear to be more tolerant, with acute NOEC values in the range of 2 to 2000 ppb. For chronic effects, the differences between small and larger arthropods are less remarkable, a stoneflies and mayflies (relatively large insects) having an NOEC value of 0.1 ppb, intermediate between *Daphnia* (0.04 ppb) and *Ceriodaphnia* (0.25 ppb). Molluscs (invertebrates including clams and snails) and worms (oligochaetes) appear to be much less sensitive to diflubenzuron.

The data on the toxicity of 4-chloroaniline to aquatic invertebrates is sparse. An acute NOEC of 0.013 mg/L is used to characterize acute risks associated with peak exposures in aquatic invertebrates and an NOEC of 0.01 mg/L from a reproduction study is used to characterize longer term risks to aquatic invertebrates.

4.3.2. Toxicity to Terrestrial Organisms
4.3.2.1. Mammals – The dose-response assessment for mammalian wildlife species is based on the same set of studies used in the human health risk assessment for diflubenzuron (Section 3.3.2) and 4-chloroaniline (Section 3.3.3).

For diflubenzuron, the most sensitive effect in experimental mammals involves toxic effects in red blood cells. The NOAEL for this endpoint in experimental mammals is 2 mg/kg/day (U.S. EPA 1997a) and is based on a study in which dogs were administered doses of 0, 2, 10, 50, or 250 mg/kg/day, 7 days/week, for 52 consecutive weeks in gelatin capsules (Greenough et al. 1985). No adverse effects, including changes in methemoglobin formation, were noted at 2 mg/kg/day. This dose will be used to characterize longer term risks to mammals. For acute exposures, the acute NOAEL of 1118 mg/kg is used. As discussed in Section 3.3.2.2, this is based on a study using a petroleum based formulation of diflubenzuron, Dimilin 2L. Because none of the estimated exposures approach a level of concern, no elaboration of the dose-response assessment is needed.

A similar approach is taken for 4-chloroaniline. The acute NOAEL is taken as 8 mg/kg. This is a very conservative approach – i.e., likely to be overly protective – because this NOAEL is from a 90 day study (Scott and Eccleston 1967). The chronic value is based on a LOAEL of 12.5 mg/kg/day from a 2-year feeding study using rats (NCI 1979). Because a NOAEL was not identified in this study, the LOAEL of 12. 5 mg/kg/day is divided by 10 to estimate a chronic

NOAEL of 1.25 mg/kg/day. This is essentially the same estimate used by U.S. EPA (1997a) in the derivation of the RfD based on the LOAEL of 12.5 mg/kg/day (Section 3.3.3.1).

4.3.2.2. Birds

4.3.2.2.1. Diflubenzuron – There appears to be relatively little difference in the acute toxicity of diflubenzuron to birds and mammals. As summarized above, the lowest acute NOAEL for mammals is 1118 mg/kg (rats dosed with Dimilin 2L in the study by Blaszcak (1997a). For birds, the lowest acute NOAEL is 2500 mg/kg from the study by Alsager and Cook (1975) in red-winged blackbirds. As detailed in Appendix 1 for mammals and Appendix 8 for birds, higher NOAEL values have been reported in other studies – i.e., up to 10,000 mg/kg for mammals (rats and mice in the study by Koopman 1977) and 5,000 mg/kg for birds (mallard ducks in the study by Roberts and Parke 1976). Analogous to the approach taken with rats, the lowest NOAEL is taken as the toxicity value for acute exposures in bird – i.e., the NOAEL of 2500 mg/kg in red-winged blackbirds from the study by Alsager and Cook 1975.

It should be noted that the variability in the acute NOAEL values does not imply any systematic differences among species but simply reflects the highest dose tested in the different experiments. Thus, the use of the lowest NOAEL rather than the highest NOAEL may be viewed as somewhat conservative. As discussed in Section 4.3.2.1, the use of the 1118 mg/kg dose for mammals is justified based on the use of a petroleum based formulation in the study by Blaszcak (1997a). The use of the lowest NOAEL for birds based on the conservative assumption that somewhat higher doses in the study by Alsager and Cook (1975) could have resulted in effects. Notwithstanding this assumption, the data are not sufficient to derive separate NOAEL values for tolerant and sensitive species because none of the available data actually demonstrated differences in sensitivity – i.e., differences in LOAEL values.

In terms of chronic toxicity, however, birds appear to be somewhat more tolerant to diflubenzuron than mammals. Based on reproduction studies, the NOEC for reproductive toxicity in birds is greater than 500 ppm – i.e., at the highest dietary concentration, no effects were noted – in mallard ducks (Beavers et al. 1990a) and bobwhite quail (Beavers et al. 1990b). Based on differences in food consumption (Appendix 4), the lowest dose in terms of mg/kg bw/day is 110 mg/kg/day from the study in quail (Beavers et al. 1990b). This is substantially above for the mammalian NOAEL of 2 mg/kg/day and the corresponding mammalian LOAEL of 10 mg/kg/day. While this suggests a difference in sensitivity between mammals and birds, the toxicity endpoints are different – i.e., effects on blood from chronic exposure in mammals and reproductive effects in birds. As noted in Appendix 1, doses as high as about 4000 mg/kg/day were not associated with reproductive effects in rats (Brooker 1995). In any event, the chronic NOAEL of 110 mg/kg/day in quail from the study by Beavers et al. (1990b) is used to characterize the risks associated with longer term exposures of birds to diflubenzuron.

4.3.2.2.2. 4-Chloroaniline – No data have been encountered on the toxicity of 4-chloroaniline to birds. For the current risk assessment, the toxicity values for 4-chloroaniline

in mammals are used as surrogates for birds. This adds uncertainty to the risk assessment for birds and this is discussed further in Section 4.4 (Risk Characterization).

4.3.2.3. Terrestrial Invertebrates

4.3.2.3.1. Diflubenzuron – Two general types of data could be used to assess dose-response relationships for terrestrial invertebrates: laboratory toxicity studies (Appendix 5) and field studies (Appendix 3a).   In most risk assessments conducted by U.S. EPA (e.g. U.S. EPA/OPP 1997a) as well as risk assessments conducted for the USDA/Forest Service, dose-response assessments for terrestrial invertebrates are based on controlled laboratory studies that are commonly conducted on the honey bee using relatively standard protocols. As indicated in Table 4-5, a different approach is used in the current risk assessment: the large number of field studies on diflubenzuron that report either effect or no effect levels are used directly for characterizing risk with exposures expressed in units of application rate.

One reason for this approach involves the disparity in experimental designs among the toxicity studies that are available which confounds quantitative comparisons of relative sensitivities among species. As discussed in Section 4.1.2.3, there is an apparently wide range of sensitivities to diflubenzuron among different invertebrate species. Based on standard toxicity tests, the honey bee is among the more tolerant species. The U.S. EPA used an LD_{50} of greater than 30 µg/bee to classify diflubenzuron as practically non-toxic to the honey bee. Taking an average weight of 0.093 g/bee or 0.000093 kg/bee (USDA/APHIS 1993) and making the very conservative assumption of 100% absorption, this would correspond to an LD_{50} greater than 322 mg/kg bw [0.03 mg/bee ÷ 0.000093 kg bw/bee = 322.58 mg/kg].   As summarized in Appendix 5, somewhat lower LD_{50} values have been reported by Chandel and Gupta (1992) – i.e., about 22 mg/kg for pupae and 53 mg/kg for third instar larvae. The gypsy moth is obviously a sensitive species, with a topical LD_{50} value of about 4 to 9 mg/kg, based on residues on vegetation (Berry et al. 1993), about a factor of 2 to 5 below the lowest LD_{50} value for the honey bee.   A similar topical LD_{50} of 1.07 mg/kg has been reported by Sinha et al. (1990) for the butterfly, *Pieris brassicae*. Somewhat lower LD_{50} values have been reported for an orthopteran – i.e., 0.31 mg/kg in *Oxya japonica* from the study by Lim and Lee (1982).   Based on topical LD_{50} values, the most sensitive species appears to be lacewing, *Chysoperla carnea*, with a reported topical LD_{50} values of 2.26 ng/insect or about 0.00226 µg/insect (Medina et al. 2003). Based on a mean body weight of 7.53 mg reported by Medina et al. (2003), this corresponds to a dose of 0.0003 µg/mg, which in turn corresponds to a dose of 0.0003 mg/g or 0.0000003 mg/kg bw. Thus, based on this LD_{50}, the lacewing would appear to be more sensitive than the gypsy moth by a factor of 13 to 30 million [4 to 9 mg/kg ÷ 0.0000003 mg/kg]. The LD_{50} value from Medina et al. (2003), however, is not really comparable to the value for the gypsy moth because the topical application to the lacewing involved direct application of diflubenzuron (in acetone) rather than a spray or contact with a contaminated surface. Thus, while the various laboratory toxicity studies could be used to construct a standard dose-response assessment for tolerant and sensitive species, there would be substantial uncertainty in the comparisons because of the diversity in experimental designs.

An alternative approach may be based on the available field studies. A summary of these studies is presented in Table 4-1 and additional details are provided in Appendix 3a. Field studies, like epidemiology studies, can be difficult to interpret because of differences in the treated site versus the control site. For example, the study by Van Den Berg (1986) on mites and collembolans is noted in Table 4-1 as providing a NOAEL in which transient or equivocal effects were noted. As detailed in Appendix 3a, Van Den Berg (1986) concluded that the effects on the mites and collembolans were insubstantial. The data, however, indicate generally fewer species over time in the treated site versus the untreated site. The author's conclusion that the effects were insubstantial is based on the fact that the populations of mites and collembolans were different at the control and treated sites prior to treatment and that the capture patterns over time for mites were highly erratic. In other words, compared to pre-treatment populations as well as the time course of population changes, the effect of diflubenzuron in this study appeared to be marginal and insubstantial. An examination of the data presented by Van Den Berg (1986) supports the conclusion that the application of diflubenzuron in this study should be classified as a NOAEL. A similar assessment may be made of the study by Martinat et al. (1993) in which changes in populations of spiders and orthopteroids (i.e., cockroaches, mantises, locusts, and crickets) were only sporadically noted over time and no consistent effect is apparent.

Despite the difficulty and uncertainty in interpreting some of the fields, the relatively large number of field studies on diflubenzuron appear to present a reasonably coherent pattern that is at least qualitatively consistent with the available toxicity data and probably a more realistic basis on which to assess risk to nontarget species. Consistent with the laboratory studies, the field studies clearly indicate that honey bees are relatively insensitive to diflubenzuron: application rates of up to 400 g/ha are not likely to affect honeybees (Table 4-5). The most sensitive species appear to be grasshoppers which may be adversely affected at an application rate of 22 g/ha. Somewhat high application rates – in the range of 30 to 35 g/ha – will adversely effect macrolepidoptera and some beneficial parasitic wasps. At the maximum application rate of considered in this risk assessment – i.e., 70 g/ha – some herbivorous insects are likely to be affected. No adverse effects in several other groups of insects are expected at this or much higher application rates, as detailed in Table 4-5.

As also noted in Section 4.1.2.3, invertebrates that do not utilize chitin are relatively insensitive to diflubenzuron. Based on soil toxicity studies, the NOEC 780 mg/kg soil for the earthworm (*Eisenia fetida*) from the study by Berends et al. (1992) is used to represent tolerant species of soil invertebrates.

4.3.2.3.2. 4-Chloroaniline

4.3.2.3.2. 4-Chloroaniline – Very little information is available on the toxicity of 4-chloroaniline to terrestrial invertebrates (WHO 2003). This is not uncommon for compounds that are not used or registered as insecticides. WHO (2003) summarizes a standard OECD study on earthworms in which the 28-day LC_{50} value was 540 mg/kg dry soil. As noted in Section 3.2, this is far higher than any concentrations of 4-chloroaniline that are likely to be found in soil.

4.3.2.4. Terrestrial Plants (Macrophytes) – As discussed in 4.1.2.4 (Hazard Identification for Terrestrial Plants), no toxicity studies have been conducted on terrestrial plants and there is no basis for asserting that adverse effects on terrestrial plants are likely from exposures to either diflubenzuron or 4-chloroaniline. Consequently, no dose-response assessments for terrestrial plants are presented in this risk assessment.

4.3.2.5. Soil Microorganisms

   4.3.2.5.1. Diflubenzuron – Diflubenzuron does not appear to be very toxic to soil microorganisms (Section 4.1.2.5). While one study (Sexstone 1995) has noted transient changes in gross microbial biomass and activity at one exposure rate (roughly equivalent to 10 g/ha), no dose-response relationship is demonstrated and the effects, if any, appear to be very minor. Consequently, this study is not used quantitatively in the dose-response assessment for soil microorganisms. For the current risk assessment, bioassays on fungi are used to identify tolerant and sensitive species – a LOEC of 50 ppm in *Pythium* for sensitive species and an NOEC of 100 ppm for tolerant species (*Aspergillus*, *Fusarium*, *Rhizopus*, *Trichoderma*) from the study by (Townshend et al. 1983). If any species of microorganisms are at risk from exposure to diflubenzuron, fungi might be considered the most likely to be susceptible because some fungi utilize chitin in their cell walls. As summarized in Table 4-2, however, the NOEC and LOEC values are several orders of magnitude higher than any plausible soil exposures.

   4.3.2.5.2. 4-Chloroaniline – The only information encountered on the microbial toxicity of 4-chloroaniline is an ED_{10} of 1000 ppm for Fe(III) reductions by upper soil (Horizon A) microorganisms (Welp and Brummer 1999). As with diflubenzuron, this concentration is far above plausible levels of soil exposure.

4.3.3. Aquatic Organisms
4.3.3.1. Fish

   4.3.3.1.1. Diflubenzuron – The toxicity data on diflubenzuron are sufficient to identify sensitive and tolerant species for both acute and chronic exposures (Table 4-4). For acute toxicity, the lowest and highest LC_{50} values will be used consistent with the data in the risk assessment presented by U.S. EPA/OPP (1997a). The LC_{50} value for sensitive fish species will be taken as 25 mg/L from the study by Johnson and Finley (1980) in yellow perch and the LC_{50} value for tolerant fish species will be taken as 500 mg/L from the study by Reiner and Parke (1975) in fathead minnow. Both of these are very protective values in that both concentrations are actually the highest concentration tested and less than 50% mortality was observed. As discussed further in Section 4.4, this protective approach has no impact on the risk assessment because the anticipated peak exposures to diflubenzuron are far below these concentrations. For longer term exposures, reproductive NOEC values will be used. The range of reported values is relatively narrow: 0.05 mg/L for mummichogs from the study by Livingston and Koenig (1977) to 0.1 mg/L for fathead minnows from the study by Cannon and Krize (1976).

   4.3.3.1.2. 4-Chloroaniline – Very little information is available on the toxicity of 4-chloroaniline to fish. As reviewed by WHO (2003), an LC_{50} value of 2.4 mg/L is reported in

bluegills and a reproductive NOEC of 0.2 mg/L in zebra fish is reported in Bresch et al. (1990). These values are used in the current risk assessment for characterizing risks to fish associated with exposures to 4-chloroaniline (Table 4-7).

4.3.3.2. Amphibians – The only information on the toxicity of diflubenzuron to amphibians comes from two field studies conducted by Pauley (1995a,b). As discussed in Section 4.1.3.2, these studies indicate a change in the diet of both terrestrial and aquatic salamanders following an application of diflubenzuron at 80g/ha. This change was secondary to changes in available food items. No data are available on the toxicity of 4-chloroaniline to amphibians. Because of the very low apparent risks to fish (Section 4.4), the limited data on effects of diflubenzuron to amphibians, and the lack of data on the effects of 4-chloroaniline to amphibians, a quantitative dose-response assessment for this group of organisms is not proposed.

4.3.3.3. Invertebrates
     4.3.3.3.1. Diflubenzuron – The toxicity values used in this risk assessment for aquatic invertebrates are summarized in Table 4-6, with the top section of this table summarizing acute toxicity values that are used to characterize risks associated with peak exposures and the bottom section of the table summarizing toxicity values used to characterize risks associated with longer term exposures. In all cases, the toxicity values are based on no-observed-effect concentrations (NOECs). This approach is somewhat different from the approach taken by U.S. EPA (1997a), in which toxicity values are based on LC_{50} values but the studies used and basic conclusions of the current risk assessment are similar to those of U.S. EPA (1997a). Diflubenzuron is very highly toxic to some aquatic invertebrates.

As with the acute toxicity to terrestrial invertebrates, the dose-response assessment can be elaborated to include several groups of invertebrates rather than simply sensitive and tolerant species. Supporting information for the acute and chronic toxicity values are given in Table 4-8 and Table 4-9, respectively, and additional information from field studies is summarized in Table 4-10. More detailed summarizes of the acute and chronic toxicity studies are given in Appendix 7 and details of a large number of field studies are given in Appendix 3b.

As summarized in Table 4-6, there is a substantial variability in the response of different groups of aquatic invertebrates to diflubenzuron. Very small arthropods – i.e, cladocerans (*Daphnia* and *Ceriodaphnia*) as well as copepods – appear to be among the most sensitive aquatic species – with acute NOEC values in the range of 0.3 to about 1 ppb (µg/L) and chronic NOEC values in the range of 0.04 to 0.25 ppb. Based on acute NOEC values, larger arthropods, including crabs and larger insects, appear to be more tolerant, with acute NOEC values in the range of 2 to 2000 ppb. In some of these assays of larger invertebrates, the short duration of the assay may be a factor in the apparently greater tolerance of larger invertebrates compared to small invertebrates. For example, Lahr et al. (2001) note that the backswimmers tested in their bioassay evidenced a NOEC of 2000 ppb but that lower NOEC values could have been evident if the organisms had been in a molting stage. This supposition is supported by chronic toxicity data (Table 4-9) in which differences between small and larger arthropods are less remarkable, with stoneflies and

mayflies (relatively large insects) having an NOEC value of 0.1 ppb, intermediate between *Daphnia* (0.04 ppb) and *Ceriodaphnia* (0.25 ppb). In the tests using stonefly and mayflies, response was characterized as an inhibition of emergence rather than pre-emergent mortality. Again, this probably relates to the inhibition of chitin synthesis by diflubenzuron. Molluscs (invertebrates including clams and snails) and worms (oligochaetes) appear to be much less sensitive to diflubenzuron.

Based on acute NOEC values, the range of sensitivities among aquatic invertebrates appears to span a factor of over 400,000 [125,000 ppb in molluscs ÷ 0.3 in *Daphnia* = 416,667] based on acute NOEC values and a factor of 8,000 [320 ppb in molluscs ÷ 0.04 in *Daphnia*] based on longer term NOEC values. These ratios are, at least to some extent, artifacts of experimental design. As summarized in Tables 4-8 and 4-9, acute and chronic NOEC and LOEC values are available for sensitive species such as daphnids. For molluscs, however, only NOEC values are available – i.e., no effects have been demonstrated in these species at the highest concentration tested.

Although there is a large number of field studies available on effects of diflubenzuron on aquatic invertebrates (Appendix 3b), these studies are not directly used in the dose-response assessments. Unlike the case with terrestrial invertebrates, application rates (e.g., g/ha) in aquatic field studies do not provide a uniform basis for comparing exposures among the different studies because the amount of diflubenzuron entering the water may and probably did vary remarkably among the different field studies based on site-specific and meteorological differences among the studies. The magnitude of possible differences is illustrated in Tables 3-2 and 3-3.

Nonetheless, some studies provide information on both application and concentrations in ambient water. An overview of these studies, summarized from Appendix 3b, is given in Table 4-10. As in the tables for standard toxicity studies, Tables 4-8 and 4-9, concentrations are given in braces [] between the species and the citation. Even these concentrations, however, are not readily comparable among studies, with some reported as peak concentrations and others as nominal or average concentrations over a given period. For example, Apperson et al. (1977) conducted a field study in which populations of cladocerans and copepods declined after an application of diflubenzuron to ponds and lakes at nominal concentrations of 2.5, 5, and 10 ppb. Actual monitored concentrations peaked at up to 32.2 ppb, however, and declined rapidly to less than 1 ppb. This type of pattern is typical in field studies in which concentrations will vary substantially both among different studies as well as over time within a single study. This probably accounts for the general pattern of field studies suggesting a higher tolerance in terms of reported concentrations than laboratory studies in which concentrations are better defined and less variable. The field studies summarized in Table 4-10, however, do support the general pattern of species sensitivity noted in the laboratory toxicity studies – i.e., small arthropods are more sensitive than larger arthropods and non-arthropod invertebrates.

Notwithstanding the limitations inherent in field studies in terms of actual exposures and temporal variations, the field studies are directly useful in risk characterization and are discussed

further in Section 4.4.   One very important feature of field studies is ability to assess population recovery, which is not typically assayed in laboratory studies. As summarized in Table 4-10, most field studies that detect adverse effects also find evidence of population recovery after application so long as the duration of the study is sufficiently long to permit the detection of recovery. This is also discussed further in the risk characterization (Section 4.4).

4.3.3.3.2. 4-Chloroaniline – The data on the toxicity of 4-chloroaniline to aquatic invertebrates is sparse, particularly when compared to the very rich data base on diflubenzuron. Notwithstanding this limitation, 4-chloroaniline appears to be much less toxic to aquatic invertebrates than diflubenzuron and the magnitude of the difference in potency can be quantified. In terms of acute toxicity to *Daphnia magna*, the 48-hour LC_{50} value for 4-chloroaniline has been reported as 0.31 mg/L (Kuhn et al 1989a), 400 times higher than the LC_{50} values of 0.0007 mg/L to 0.00075 mg/L for diflubenzuron (Corry et al. 1995; Kuijpers 1988; Majori et al. 1984). The corresponding NOEC for 4-chloroaniline is 0.013 mg/L (Kuhn et al 1989a), 40 times higher than the acute NOEC of 0.0003 mg/L for diflubenzuron (Corry et al. 1995).

Similarly, the chronic NOEC in *Daphnia magna* for 4-chloroaniline in a standard reproduction study is 0.01 mg/L (Kuhn et al 1989b). This is a factor of 250 times higher than the corresponding value of 0.00004 mg/L in *Daphnia magna* reported by Surprenant (1988).

As summarized in Table 4-7 (toxicity values for 4-chloroaniline), the acute NOEC of 0.013 mg/L (Kuhn et al 1989a) is used to characterize acute risks to aquatic invertebrates and the NOEC of 0.01 mg/L for reproductive effects (Kuhn et al 1989b) is used to characterize longer term risks to aquatic invertebrates.

4.3.3.4. Aquatic Plants
4.3.3.4.1. Diflubenzuron – Compared to aquatic invertebrates, relatively little information is available on the toxicity of diflubenzuron to aquatic plants (Section 4.1.3.4 and Appendix 8). The lowest reported effect is a decrease in periphyton at a concentration 7.0 µg/L in littoral enclosures (Moffett 1995). As noted in Section 4.1.3.4 and Appendix 8, Moffett (1995) attributed this change to a decrease in the population density of zooplankton grazers. This conclusion seems reasonable and is supported by standard plant toxicity studies reporting no effects at concentrations of up to 380 µg/L (Booth and Ferrell 1977; Thompson and Swigert 1993a,b,c). For assessing the risks of direct toxic effects on terrestrial plants, a NOEC of 45 µg/L will be used for possibly sensitive species (*Selenastrum capricornutum* in the study by Hansen and Garton 1982a) and a NOEC of 380 µg/L (*Navicula pelliculosa* in the study by Thompson and Swigert 1993c) will be used for apparently tolerant species. Since no LOEC values are available for any species of aquatic plants, these different NOEC values may simply reflect differences in the highest dose tested in the respective experiments rather than true differences in species sensitivity to diflubenzuron.

4.3.3.4.2. 4-Chloroaniline – The only information encountered on the toxicity of 4-chloroaniline is summarized in WHO (2003) from two publications in the German literature (Schmidt 1989; Schmidt and Schnabl 1988). Based on this information, 4-chloroaniline appears to be somewhat more toxic to aquatic plants than diflubenzuron.   While WHO (2003) does not report NOEC values for 4-chloroaniline, an EC_{10} of 0.02 mg/L for cell multiplication in *Scenedesmus subspicatus*, a species of green algae, will be used as surrogate NOEC.

4.3.3.5. *Microorganisms* (excluding algae)

4.3.3.5.1. Diflubenzuron – Very little information is available on the toxicity of either diflubenzuron or 4-chloroaniline to aquatic microorganisms. As summarized in Section 4.1.3.5, marginal and transient effects on microbial decomposition and respiration have been noted at 50 µg/L and 50,000 µg/L (Kreutzweiser et al. 2001). Because of the insubstantial nature of the effects and the lack of a marked dose-response relationship, the concentration of 50 µg/L is used as a NOEC for aquatic microorganisms in Table 4-4.

4.3.3.5.2. 4-Chloroaniline – The only information on 4-chloroaniline is the results of a assay for bioluminescence with *Photobacterium phosphoreum* in which the 30-minute EC_{50} for the inhibition of bioluminescence was 5.1 mg/L (Ribo and Kaiser 1984). While the utility of this type of assay for risk characterization may be marginal, it is the only information available and is included in Table 4-7 and used for the risk characterization of 4-chloroaniline.

4.4. RISK CHARACTERIZATION
4.4.1. Overview
While the data base supporting the ecological risk assessment of diflubenzuron is large and complex, the risk characterization is relatively simple. Diflubenzuron is an effective insecticide and effects on some nontarget terrestrial insects are likely at application rates that are used to control the gypsy moth. Species at greatest risk include grasshoppers, various macrolepidoptera (including the gypsy moth), other herbivorous insects, and some beneficial predators of the gypsy moth. These species are at risk because of the mode of action of diflubenzuron (i.e., inhibition of chitin) and the behavior of the sensitive insects (the consumption of contaminated vegetation or predation on the gypsy moth). Some aquatic invertebrates may also be at risk but the risks appear to be less than risks to terrestrial insects. The risk characterization for aquatic invertebrates is highly dependant on site-specific conditions. In areas in which water contamination is likely to be minimal, no or only marginal effects are expected. During applications in which drift or direct deposition is not controlled well or in areas in which soil losses from runoff and sediment are likely, acute effects on some aquatic invertebrates are plausible and longer term effects on sensitive species could occur.

Direct effects of diflubenzuron on other groups of organisms – i.e., mammals, birds, amphibians, fish, terrestrial and aquatic plants, microorganisms, and non-arthropod invertebrates – do not appear to be plausible. Secondary effects in some nontarget species could occur. The most common secondary effects will be seen in and associated with animals that consume either the the gypsy moth or other invertebrates that may be adversely affected by diflubenzuron. The most common secondary effect will be a change in prey items that are consumed. Changes in feeding territory and prey items as well as reductions in body fat are likely to be transient.

There is no indication that 4-chloroaniline formed from the degradation of diflubenzuron will have an adverse effects on any species.

4.4.2. Terrestrial Organisms
4.4.2.1. Terrestrial Vertebrates – The risk characterizations for terrestrial vertebrates are essentially identical for both diflubenzuron and 4-chloroaniline. At the highest application rate of diflubenzuron that would be used in USDA programs, risks to mammals and birds are far below a level of concern. The quantitative risk characterization for terrestrial vertebrates (mammals and birds) is summarized in Worksheet G02a in the diflubenzuron worksheets (Supplement 1) and Worksheet G02 in the 4-chloroaniline worksheets (Supplement 2). The risk characterization is based on the estimates of exposure summarized in Section 4.2.3 and the toxicity values for diflubenzuron (Table 4-4) and 4-chloroaniline (Table 4-7) that were derived in Section 4.3.2.

The highest hazard quotient (HQ) for diflubenzuron is 0.2, the value associated with the upper range of exposure from the longer term consumption of contaminated vegetation in the treated area by a large mammal. As discussed in Section 4.2.2, this exposure scenario is based on the consumption of contaminated grass by a large mammal. For the gypsy moth program, this is an

extremely conservative scenario in that most large wildlife mammals will not consume grass as an exclusive or even predominant proportion of their diet (exceptions being elk and some livestock animals). In addition, this scenario assumes that the grass is directly sprayed. In the application of diflubenzuron, canopy interception would reduce residues on grass in most circumstances. Other hazard quotients for diflubenzuron are below a level of concern by factors of 50 (the upper range HQ of 0.02 for the consumption of contaminated fish by a predatory bird) to 1 in one billion (the lower range HQ for the consumption of contaminated water by a small mammal).

The highest risk quotient for chloroaniline is 0.02, associated with the consumption of contaminated water by a small mammal. As discussed in Section 3.2.3.4, these peak exposures may occur months after the application of diflubenzuron and the concentrations of 4-chloroaniline in water are likely to vary substantially with different soils as well as rainfall rates. The peak concentrations of 4-chloroaniline are based on very conservative and perhaps extreme assumptions and the very low of hazard quotient of 0.02 – i.e., below the level of concern by a factor of 50 – indicates that there is no plausible basis for asserting that such exposures would be hazardous.

This risk characterization for terrestrial vertebrates is consistent with the risk characterization by U.S. EPA (1997a) as well as field studies which indicate a lack of adverse effects on terrestrial vertebrates after applications of diflubenzuron (Sections 4.1.2.1 and 4.1.2.2. and Appendix 3a). No toxic effects are likely to be seen in mammals or birds.

The most common secondary effects will be seen in and associated with vertebrates that consume either the target species (the gypsy moth) or other invertebrates that may be adversely affected by diflubenzuron (see Section 4.4.2.2.1). For such vertebrates, the most common secondary effect will be a change in prey items that are consumed.

4.4.2.2. Terrestrial Invertebrates

4.4.2.2.1. Diflubenzuron – While risks to terrestrial vertebrates are implausible, risks to some terrestrial invertebrates are virtually certain (Worksheet G02b, Supplement 1). At an application rate of 70 g/ha, adverse effects – i.e., mortality and decreases in populations – have been demonstrated in field studies for grasshoppers, various macrolepidoptera (including the gypsy moth), some mandibulate herbivores, and some beneficial predators to the gypsy moth. Effects on some beneficial predators may be secondary but at least in one species, *Apanteles melanoscelus*, a wasp that is a parasite on the gypsy moth, the effect appears to be due to direct toxicity (Madrid and Stewart1981). Effects in the same species are likely to be seen at lower application rates that may be used in USDA programs – i.e., 35 g/ha. For effects in these sensitive groups to be avoided, the application rate would need to be below about 2 g/ha [70 g/ha from Worksheet G02b divided by the HQ of 32 for the grasshopper]. This damage to non-target species appears to be unavoidable given the mode of action of diflubenzuron (i.e., inhibition of chitin) and the behavior of the sensitive insects (the consumption of contaminated vegetation or predation on the gypsy moth).

Most other insect groups are not likely to be affected at least directly. Some secondary effects associated with changes in available prey may be noted. As with most secondary effects, the changes in habitat or prey items are likely to be reversible. In other words, changes will be transient and populations will generally recover (e.g., Catangui et al. 1996).

4.4.2.2.2. 4-Chloroaniline – Very little information is available on the toxicity of 4-chloroaniline to invertebrates. One bioassay in earthworms reports an LC_{50} value of 540 mg/kg soil. The maximum concentration of 4-chloroaniline in soil is estimated at 0.0026 ppm (Table 4-3). The resulting HQ is 4.8×10^{-6}, below the level of concern by over 200,000. No data are available on the toxicity of 4-chloroaniline to other terrestrial vertebrates and risks cannot be quantified. Given the relatively low risks of 4-chloroaniline in aquatic invertebrates (4.4.3.2.2) as well as other organisms, there is no basis for asserting that substantial risks are plausible, particularly when compared to clear risks associated with diflubenzuron.

4.4.2.3. Terrestrial Plants and Microorganisms – No quantitative risk assessment to terrestrial plants is made for either diflubenzuron or 4-chloroaniline. As discussed in Section 4.1.2.4, there are no data on the phytotoxicity of either compound. This lack of data, however, adds no substantial uncertainty to this risk assessment. Diflubenzuron has been extensively tested in both the laboratory and field studies for efficacy in the protection of terrestrial plants from insect pests. If diflubenzuron were toxic to plants at applications at or substantially above those used to control the gypsy moth, it is likely that reports of such phytotoxicity would be noted. No such reports have been encountered (Appendix 3a and Appendix 8).

Limited information is available on the toxicity of diflubenzuron and 4-chloroaniline to soil microorganisms. As summarized in Worksheet G02b for diflubenzuron (Supplement 1), exposures of soil microorganisms to diflubenzuron are likely to be below a level of concern for sensitive species by a factor of over 600 at the upper range of plausible exposure – i.e., an HQ of 0.0016. For 4-chloroaniline, the toxicity value for microorganisms is 1000 ppm. As noted above, the highest estimated peak concentration of 4-chloroaniline in soil is 0.0026 ppm (Table 4-3). The resulting HQ is 2.6×10^{-6}, below the level of concern by over 350,000.

4.4.3. Aquatic Organisms
4.4.3.1. Aquatic Vertebrates – As with terrestrial vertebrates, the risk assessment for fish is unequivocal. There is no indication that diflubenzuron or 4-chloroaniline associated with the degradation of diflubenzuron will approach a level of concern.

The highest hazard quotient for diflubenzuron is 0.002 – i.e., longer term exposures to sensitive fish species (Worksheet G03b in Supplement 1). This is below the level of concern by a factor of 500. The toxicity of diflubenzuron has been assayed in relatively few fish species and it is likely that the most sensitive species of fish has not been identified. Nonetheless, there is no basis for asserting that species variability will encompass the factor of 500 associated with the highest HQ for diflubenzuron.

The risk characterization for 4-chloroaniline is virtually identical. The highest hazard quotient is 0.001. Below the level of concern by a factor of 1000 (Worksheet G03, Supplement 2).

4.4.3.2. Aquatic Invertebrates

4.4.3.2.1. Diflubenzuron – As noted by U.S. EPA (1997a), risks to aquatic invertebrates in some applications of diflubenzuron may be substantial – i.e., direct applications to standing bodies of water for mosquito control and forestry uses involving direct applications to bogs, swamps or other standing bodies of water (U.S. EPA 1997a, p. 64). These types of applications, however, are not used in and are thus not relevant to USDA programs for the control of the gypsy moth.

In USDA programs for control of the gypsy moth, risks to aquatic invertebrates appears to be substantially less than risks to terrestrial invertebrates. As noted in Section 2.3, USDA will use a 100 to 500 foot buffer between the application site of diflubenzuron and bodies of open water. While it is possible that small streams could be over-sprayed in aerial applications if the stream is not visible from the air, the covering foliar canopy would intercept some of the diflubenzuron which would in turn reduce the initial concentrations in stream water.

Based on the exposure assessments conducted in this risk assessment, which are consistent with several other exposure assessments as well as a number of relevant monitoring studies (Table 3-7), only the most sensitive species of aquatic invertebrates are likely to be adversely affected based on central estimates of plausible peak exposures. The central estimate of the hazard quotient for sensitive daphnids is only 1.3 (Worksheet G03a, Supplement 1). Typically, hazard quotients are rounded to a single significant digit. Thus, this hazard quotient reaches but does not exceed a level of concern. Based on central estimates of longer term exposures, all hazard quotients are less than 1 (Worksheets G03b, Supplement 1).

At the upper ranges of plausible peak exposures, the level of concern is reached for crabs (HQ=1), modestly exceeded for *Ceriodaphnia* and copepods (HQ=2), and exceeded by a factor of 5 for *Daphnia*. For *Daphnia*, LC_{50} values are only modestly above the NOEC (Table 4-8) and substantial mortality in these species would be plausible. At the upper range of longer term exposures, the hazard quotient exceeds a value of 1 only for *Daphnia* – i.e., HQ=3. This is in the range in which longer term effects on *Daphnia* productivity would be expected and such effects have been observed in field studies (Ali and Mulla 1978b).

Thus, based on the available toxicity data and dose response assessment, the risk characterization for aquatic invertebrates is highly dependant on site-specific conditions. In areas in which water contamination is likely to be minimal – i.e., areas with relatively low rainfall and areas in which drift can be controlled and runoff is limited – it is likely that no or only minimal effects would be observed (e.g., the field study by Ali et al. 1988). During applications in which drift or direct deposition is not controlled well or in areas in which soil losses from runoff and sediment are likely, acute effects on some aquatic invertebrates are plausible and longer term effects on sensitive species could occur.

That any of these effects would result in substantial secondary effects does not seem likely. A large number of field studies are available on diflubenzuron (Appendix 3b) that indicate direct effects on several species of invertebrates at concentrations in water that are above those that would be encountered in many applications for the control of the gypsy moth (see Section 4.1.3.3 for discussion). In addition, the only studies that suggest substantial secondary effects – such as decreased growth in fish – are litoral enclosure studies (Moffett and Tanner 1995; Tanner and Moffett 1995) in which fish were limited in their ability to seek prey. None of the field studies involving free-ranging fish have reported secondary effects other than a change in prey that are consumed.

   4.4.3.2.2. 4-Chloroaniline – The risks to aquatic invertebrates associated with 4-chloroaniline are insubstantial relative to the risks associated with diflubenzuron. The highest hazard quotient is 0.2, associated with peak exposures to 4-chloroaniline in water.

4.4.3.3. Aquatic Plants and Microorganisms – Risks to aquatic plants and microorganisms appear to be low. There is essentially no identifiable risk associated with diflubenzuron. The highest hazard quotient is 0.04 and is associated with peak exposures to sensitive aquatic plants (Worksheet G03a, Supplement 1). Peak risks associated with 4-chloroaniline are somewhat higher, 0.2, the HQ associated with peak exposures to aquatic plants (Worksheet G03, Supplement 2).

A more plausible risk to aquatic plants may involve secondary effects – increased algal populations – associated with mortality in aquatic grazers such as Cladocerans. This effect has been noted in the mesocosm study by Boyle et al. (1996) . Apperson et al. (1977) noted a decrease in the concentration of a blue-green algae (*Anabaena* species) but no effect on diatoms or green algae. It is unclear if the effect was a primary, secondary, or incidental effect.

5. REFERENCES

Ables JR; West RP; Shepard M. 1975. Response of the house fly and its parasitoids to Dimilin (TH-6040). Journal of Economic Entomology. 68(5): 622-624. Cited in USDA 1995.

Ables JR; Jones SL; Bee MJ. 1977. Effect of diflubenzuron on beneficial arthropods associated with cotton. Southwestern Entomologist 2(2):66-72. Cited in USDA 1995.

Ahmad ME. 1994. Effect of Dimilin on growth, moulting, metamorphosis, reproduction and progeny development of *Dysdercus cingulatus*. Journal of Advanced Zoology. 15(1): 44-48.

Alho CJR; Vieira LM. 1997. Fish and wildlife resources in the pantanal wetlands of Brazil and potential disturbances from the release of environmental contaminants. Environmental Toxicology and Chemistry. 16(1): 71-74.

Ali A; Lord J. 1980. Impact of experimental insect growth regulators on some nontarget aquatic invertebrates. Mosq. News 40(4): 564-571.

Ali A; Mulla MS. 1978a. Effects of Chironomid larvicides and diflubenzuron on nontarget invertebrates in residential - recreational lakes. Environ. Entomol. 7(1): 21-27.

Ali A; Mulla MS. 1978b. Impact of the insect growth regulator diflubenzuron on invertebrates in a residential-recreational lake. Arch Environ Contam Toxicol. 7(4): 483-491.

Ali A; Nigg HN; Stamper JH; Kok-Yokomi ML; Weaver M. 1988. Diflubenzuron application to citrus and its impact on invertebrates in an adjacent pond. Bull Environ Contam Toxicol. 41(5): 781-790.

Ali A; Chowdhury MA; Hossain MI; ul-Ameen M; Habiba DB; Aslam A FM. 1999. Laboratory evaluation of selected larvicides and insect growth regulators against field-collected *Culex quinquefasciatus* larvae from urban Dhaka, Bangladesh. Journal of the American Mosquito Control Association. 15(1): 43-47.

Alsager DF; Cook DA. 1975. Acute Oral Toxicity Studies (LD$_{50}$) of TH6040 Insecticide to Red Winged Blackbirds (*Agelaius phoeniceus* : CBSC No. TR-112-75. (Unpublished study received Feb 10, 1976 under 6G1744; prepared by Canadian Bio-Scientific Consultants, Ltd. and Univ. of Alberta, Dept. of Pharmacology, submitted by Thompson-Hayward Chemical Co., Kansas City, Kans.; CDL:094974-G) MRID 00038614.

Anderson DW; Elliott RH. 1982. Efficacy of diflubenzuron against the codling moth, *Laspeyresia pomonella* (Lepidoptera: Olethreutidae), and impact on orchard mites. Canadian Entomologist 114:733-737. Cited in USDA 1995.

Andre J. 1996. Dermal Absorption of (Carbon[14])-Diflubenzuron by Male Sprague-Dawley Rats: Lab Project Number: 6615-95-0275-AM-001: 6615-95-0275-AM-000-001. Unpublished study prepared by Ricerca, Inc. 120 p. MRID 44053101.

Anton FA; Cuadra LM; Gutierrez P; Laborda E; Laborada P. 1993. Degradational behavior of the pesticides glyphosate and diflubenzuron in water. Bull Environ Contam Toxicol. 51: 881-888.

Apperson C; Schaefer C; Colwell A. 1977. Effects of Diflubenzuron on *Chaoborus astictopus* Dyar and Shannon (Diptera: Chaoboridae) and Nontarget Organisms, and Persistence of Diflubenzuron in Lentic Habitats. (Unpublished study received Jul 31, 1978 under 148-1259; prepared by North Carolin. (California), Mosquito Abatement District, submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL:234511-AC). MRID 00099897.

Apperson CS; Schaefer CH; Colwell AE; Werner GH; Anderson NL; Dupras Ef Jr; Longanecker DR. 1978. Effects of diflubenzuron on *Chaoborus astictopus* and nontarget organisms and persistence of diflubenzuron in lentic habitats. J. Econom. Entomol. 71(3): 521-527.

Arnold DA. 1974. Mutagenic study with TH 6040 in albino mice. Industrial Bio-test Laboratories. (Cited in WHO 2001).

{Atkins et al. 1974} Atkins EL; Greywood-Hale EA; Macdonald RL; et al. (1974) Effect of Pesticides on Apiculture: 1974 Annual Report: Project No. 1499. (Unpublished study received October 21, 1976 under 6F1696; prepared by University of California -- Riverside, Agricultural Experiment Station, Department of Entomology, Citrus Research Center, submitted by E.I. duPont de Nemours & Company, Inc., Wilmington, Del., CDL:095326-K). MRID 00040601. Summarized in U.S. EPA 1997a.

Atkins EL; Kellum D; Atkins KW. 1981. Reducing pesticide hazards to honey bees: Mortality prediction techniques and integrated management strategies. Leaflet 2883. University of California, Division of Agricultural Sciences, Riverside, CA. Cited in USDA 1995.

ATSDR (Agency for Toxic Substances and Disease Registry). 2003. Toxicological Profile for Fuel Oils. ATSDR ToxProfiles on CD-ROM. Available from U.S. Department of Health and Human Services, Public Health Service, ATSDR, Division of Toxicology. Http://www.atsdr.cdc.gov/

Babic S; Kastelan-Macan M; Petrovic M. 1998a. Determination of agrochemical combinations in spiked soil samples. Water Science and Technology. 37(8): 243-250.

Babic S; Petrovic M; Kastelan-Macan M. 1998b. Ultrasonic solvent extraction of pesticides from soil. Journal of Chromatography A. 823(1-2): 3-9.

Baishya RL; Hazarika LK. 1996. Effect of methoprene and diflubenzuron on water, lipid, protein and chitin content of *Dicladispa armigera* (Coleoptera: chrysomelidae). Entomon. 21(1): 7-11.

Bajwa WI; Aliniazee MT. 2001. Spider fauna in apple ecosystem of western Oregon and its field susceptibility to chemical and microbial insecticides. J Econ Entomol. 94(1):68-75.

Barker RY; Taber S. 1977. Effects of diflubenzuron fed to caged honey bees. Environ Entomol, 6: 167-168. Summarized in WHO 1997.

Barker RJ; Waller GD. 1978. Effects of diflubenzuron wettable powder on caged honeybee colonies. Environ Enotomology. 7: 534-535.

Barretto OC; Halsman MW; Nonoyama K; Tamigaki M; Maspes V. 1984. Congenital deficiency of erythrocyte NADH-dependent methemoglobin reductase (diaphorase). Sangre (Barc) 29(1): 62-66. Cited in USDA 1995.

Barrows EM; Wolf SS; Lynch DM. 1994. Diflubenzuron effect on yellowjacket (Hymenoptera: vespidae) worker numbers in a central Appalachian broadleaf forest. Journal of Economic Entomology. 87(6): 1488-1493.

Barrows EM. 1995. Chapter 8. Pollinating Insects – Native Species. pp. 66-76 in: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Beavers J; Corbitt A; Hawrot R; et al. 1990a. Diflubenzuron: A One-Generation Reproduction Study with the Mallard (*Anas platyrhynchos*). Lab Project Number: 225-103: C.303.40.014: 56645 /07/90. Unpublished study prepared by Wildlife International Ltd. 140 p. MRID 41668001.

Beavers J; Corbitt A; Hawrot R; et al. 1990b. Diflubenzuron: A One-Generation Reproduction Study with the Bobwhite (*Colinus virginianus*). Lab Project Number: 225-102: C.303.40.013. Unpublished study prepared by Wildlife International Ltd. 143 p. MRID 41668002.

Beavers J; Corbitt A; Hawrot R; et al. 1990c. Diflubenzuron: A One-Generation Reproduction Study with the Mallard. (*Anas platyrhynchos*): Lab Project Number: 225-103: C.303.40.014: 56645 /07/90. Unpublished study prepared by Wildlife International Ltd. 140 p. MRID 41668001.

Beavers J; Corbitt A; Hawrot R; et al. 1990d. Diflubenzuron: A One-Generation Reproduction Study with the Bobwhite. (*Colinus virginianus*): Lab Project Number: 225-102: C.303.40.013. Unpub- lished study prepared by Wildlife International Ltd. 143 p. MRID 41668002.

Beevi, S.P.; Dale, D., 1984. Sterilant and ovicidal actions of diflubenzuron on the rice swarming caterpillar. Pesticides (Bombay) 18(10):54-55. Cited in USDA 1995.

Bell JL; Whitmore RC. 1997. Bird populations and habitat in *Bacillus thuringiensis* and Dimilin-treated and untreated areas of hardwood forest. American Midland Naturalist. 137(2): 239-250.

Berczy ZS; Cobb LM; Street AE. 1975. Subacute Inhalation Toxicity to the Rat of Du 112307 Insecticide Powder (Evaluation of Methaemoglobinaemia): PDR197/741013. (Unpublished study received Feb 10, 1976 under 6G1744; prepared by Huntingdon Research Centre, England, submitted by Thompson-Hayward Chemical Co., Kansas City, Kans.; CDL:094965-B). MRID 00044325.

Berry RE; Noldenke AF; Miller JC; Wernz JG. 1993. Toxicity of diflubenzuron in larvae of gypsy moth (Lepidoptera: lymantriidae): effects of host plant. J Econ Entomol. 86: 809-814.

Biddinger DJ; Hull LA. 1999. Sublethal effects of selected insecticides on growth and reproduction of a laboratory susceptible strain of tufted apple bud moth (Lepidoptera: tortricidae). Journal of Economic Entomology. 92(2): 314-324.

Bionomics--EG&G Incorporated. 1975. The Acute and Subchronic Toxicity of R-20458, Altosid and TH-6040 to the Grass Shrimp, *Palaemonetes puglio*. Final rept. (Unpublished study received Feb 10, 1976 under 6G1744; submitted by Thompson-Hayward Chemical Co., Kansas City, Kans.; CDL:094974-D). MRID 00038612.

Birdsong R. 1965. Field Test of Dimilin on Non-target Organisms in Virginia: File #179. Final rept. (Unpublished study received July 19, 1976 under 148-1262; prepared by Environmental Consultants, Inc., submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL:096204-H). MRID 00099791.

Blaszcak D. 1997a. Acute Oral Toxicity Study with Dimilin 2L in Rats: Final Report: Lab Project Number: 96-1493. Unpublished study prepared by Huntingdon Life Sciences. 22 p. MRID 44574504.

Blaszcak D. 1997b. Acute Dermal Toxicity Study with Dimilin 2L in Rabbits: Final Report: Lab Project Number: 96-1494. Unpublished study prepared by Huntingdon Life Sciences. 22 p. MRID 44574505.

Blaszcak D. 1997c. Primary Eye Irritation Study with Dimilin 2L in Rabbits: Final Report: Lab Project Number: 96-1496. Unpublished study prepared by Huntingdon Life Sciences. 26 p. MRID 44574507.

Blaszcak D. 1997d. Primary Dermal Irritation Study with Dimilin 2L in Rabbits: Final Report: Lab Project Number: 96-1495. Unpublished study prepared by Huntingdon Life Sciences. 20 p. MRID 44574508.

Blaszcak D. 1997e. Closed-Patch Repeated Insult Dermal Sensitization Study with Dimilin 2L in Guinea Pigs. (Buehler Method): Final Report: Lab Project Number: 96-1497. Unpublished study prepared by Huntingdon Life Sciences. 30 p. MRID 44574509.

Blumberg, A.Y. 1986. Survey of aquatic, soil and soil surface invertebrate fauna in a North Carolina forest (pre- and post application of Dimilin WP-25). Duphar B.V., Crop Protection Division, Department of Biochemistry, Graveland, the Netherlands. 42p. Cited in USDA 1995.

Bollag J; Blattmann P; Laanio T. 1978. Adsorption and transformation of four substituted anilines in soil. J Agric Food Chem. 26(6): 1302-1305 MRID 40660602.

Booth GM. 1975. The Impact of Dimilin W-25 on Non-target Invertebrates in Ponds Located in Salt Lake County, Utah. Final rept. (Unpublished study received Feb 10, 1976 under 6G1744; prepared by Brigham Young Univ., Dept. of Zoology, submitted by Thompson-Hayward Chemical Co.; Kansas City, Kans.; CDL:094969-H). MRID 00038213.

Booth GM. 1978. Dimilin and the environment. In: Dimilin chitin inhibitor breakthrough in pest control. Agri-Fieldman and Consultant, Kansas City, KS. Cited in USDA 1995.

Booth G; Ferrell D. 1977. Degradation of Dimilin by Aquatic Foodwebs. (Unpublished study received Jul 31, 1978 under 148- 1259; submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL:234511-K). MRID 00099884.

Booth SR; Riedl H. 1996. Diflubenzuron-based management of the pear pest complex in commercial orchards of the hood river valley in oregon. J Econ Entomol. 89: 621-630.

Booth G; Johnson S; Human D; et al. 1977. The Effect of Diflubenzuron on the Reproduction of Bobwhite Quail. (Unpublished study received Feb 6, 1978 under 148-1259; prepared by Brighan Young Univ., Depts. of Statistics and Zoology and Univ. of Illinois, School of Life Sciences, submitted by Thompson- Hayward Chemical Co., Kansas City, KS; CDL:096787-J). MRID 0.

Bouvier JC; Boivin T; Beslay D; Sauphanor B. 2002. Age-dependent response to insecticides and enzymatic variation in susceptible and resistant codling moth larvae. Arch Insect Biochem Physiol. 51(2):55-66.

Boxenbaum J; D'Souza R. 1990. Interspecies pharmacokinetic scaling, biological design and neoteny. Adv Drug Res. 19: 139-195.

Boyle TP; Farichild JF; Robinson-Wilson EF. 1996. Ecological restructuring in experimental aquatic mesocosms due to the application of diflubenzuron. Environ Toxicol Chem. 15: 1806-1814.

Berends, A.G.; Thus, J.L.G., 1992. The acute toxicity of Dimilin WP-25 to the earthworm Eisenia fetida. Laboratory project C.303.51.014, Solvay Duphar B.V., Environmental Research Department, Graveland, the Netherlands. Cited in USDA 1995.

Berends, A.G.; Thus, J.L.G.; Jansen, W.A.J. 1992a. The acute toxicity of diflubenzuron to the earthworm Eisenia fetida. Laboratory project C.303.51.011, Solvay Duphar B.V., Environmental Research Department, Graveland, the Netherlands. Cited in USDA 1995.

Berends, A.G; Thus, J.L.G.; Jansen, W.A.J. 1992b. Acute toxicity of diflubenzuron to the earthworm *Eisenia fetida*. Duphar B. V. Internal Document No. 56635/22/92 16 pp. Cited in USDA 1995.

Bradberry SM. 2003. Occupational methaemoglobinaemia. Mechanisms of production, features, diagnosis and management including the use of methylene blue. Toxicol Rev. 22(1):13-27.

Bradt PA; Williams JA. 1990. Response of Hydropsychidae (Insecta: Trichoptera) larvae to diflubenzuron. Journal of the Pennsylvania Academy of Science 64(1):19-22. Cited in USDA 1995.

Bresch H; Beck H; Ehlermann D; Schlaszus H; Urbanek M. 1990. A long-term toxicity test comprising reproduction and growth of zebrafish with 4-chloroaniline. Archives of Environmental Contamination and Toxicology, 19:419–427. Summarized in WHO 2003.

Breteler R. 1987. Chronic Toxicity of Diflubenzuron to Mysid Shrimp. (*Mysidopsis bahia*), Part II, Supplemental Studies: Laboratory Project ID: 11493-0886-6100-530 and #BW-87-5-2400. Un- published compilation prepared by Springborn Bionomics, Inc. 44 p. MRID 40237501.

Broadbent, A.B.; Pree, D.J. 1984a. Effects of diflubenzuron and BAY SIR 8514 on beneficial insects associated with peach. Environmental Entomology 13(1):133-136. Cited in USDA 1995.

Broadbent, A.B.; Pree, D.J. 1984b. Effects of diflubenzuron and BAY SIR 8514 on the oriental fruit moth, *Grapholitha molesta* (Busck), and the oblique-banded leafroller, *Choristoneura rosaceana* (Harris). Journal of Economic Entomology 77:194-197. Cited in USDA 1995.

Brooker A. 1995. Diflubenzuron Technical: The Effect on Reproductive Function of Two Generations in the Rat: Lab Project Number: PDR 569: 56345/83/94: PDR 569/932539. Unpublished study prepared by Huntingdon Research Centre Ltd. 416 p. MRID 43578301.

Brown MW; Repicio NC. 1981. Effect of diflubenzuron on the Gypsy moth egg parasite *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae). Melsheimer Entomological Series (31):1-7. Cited in USDA 1995.

Brusick DJ; Weir RJ 1977a. Evaluation of diflubenzuron: in vitro malignant transformation in BALB/3T3 cells. Litton Bionectics Project No. 2688. (Unpublished). (Cited in WHO 2001).

{Brusick et al. 1977b} Brusick DJ; Weir RJ. 1977b. Evaluation of diflubenzuron: unscheduled DNA synthesis in WI-38 cells. Litton Bionectics Project No. 2688. (Unpublished). (Cited in WHO 2001).

{Brusick et al. 1977c} Brusick, DJ; Weir RJ. 1977c. Mutagenicity evaluation of diflubenzuron technical, batch Fl44/60521. Litton Bionetics Project No. 2683. (Unpublished). (Cited in WHO 2001).

{Büchi and Jossi 1979} Büchi, R.; Jossi, W. 1979. Über die Wirkung des Wachstumsregulators Dimilin auf den Maikäfer Melolontha melolontha L. und den Blackenkäfer Gastroidea viridula DEG. Mitteilungen der Schweizerischen Entomologischen Gesellschaft 52(1):75-82. Cited in USDA 1995.

Buckner CH; McLeod BB; Kingsbury PD. 1975. The Effect of an Experimental Application of Dimilin[®] upon Selected Forest Fauna: Report CC-X-97. (Canada--Environment, Forestry Service, Chemical Control Research Institute; unpublished study; CDL:096232-K). MRID 00071210. Also summarized in WHO 1997.

Buisman P; Verhaar L. 1985. Residues og Duflubenzuron in Feed, Eggs, Manure and Liver from a Feed-through Trial in Chicken Carried Out with Diets Containing VC90 and Technical Difluben- zuron. (Muiden, The Netherlands): Laboratory Project ID 84-11. Unpublished study prepared by Duphar B.V. 27 p. MRID 40424601.

Bull, D.L.; Coleman, R.J. 1985. Effects of Pesticides on Trichogramma spp. Southwestern Entomologist Supplement 0(8):156-168. Cited in USDA 1995.

Burdock G. 1984. Oncogenicity Study in Rats: Final Report: Pro- ject No. 553-122. Unpublished study prepared by Hazleton Lab- oratories America, Inc. 4230 p. MRID 00145467.

Burdock GA; Wentz KL; Purvis D; et al. 1980. Subchronic Dietary Toxicity Study in Rats: Diflubenzuron: Project No. 553- 119. Final rept. (Unpublished study received Nov 21, 1980 under 148-1268; prepared by Hazleton Laboratories America, Inc., submitted by Thompson-Hayward Chemical Co., Kansas City, Kans.; CDL:243807-A; 243808, 243809; 243805; 243806). MRID 00064550.

Burgess D. 1989. Uptake, Depuration and Bioconcentration of [carbon^{14}-diflubenzuron by Bluegill Sunfish (Lepomis macrochirus). Lab Project Number: 37511. Unpublished study prepared by Analytical Bio-Chemistry Laboratories, Inc. 41 p. MRID 42258401.

Butler L., 1993. Dimilin impact on Lepidoptera and other canopy arthropods: preliminary results. 1993 USDA Interagency Gypsy Moth Research Forum: 28. Cited in USDA 1995.

Butler L. 1995. Chapter 7. Canopy Arthropods. pp. 53-65 in: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Butler L; Chrislip GA; Kondo VA; Townsend EC. 1997. Effect of diflubenzuron on nontarget canopy arthropods in closed, deciduous watersheds in a central Appalachian forest. J Econ Entomol. 90: 784-794.

C&P Press (Chemical and Pharmaceutical Press). 2004. Greenbook.net. Product Labels and Material Safety Data Sheets for Dimilin 4L, Dimilin 25W, and other formulations of diflubenzuron. Available at: http://www.greenbook.net/ .

Calabrese, EJ. 1991. Principles of animal extrapolation. Lewis Publishers. Chelsea, MI.

Calabrese EJ; Baldwin LA. 1993. Performing Ecological Risk Assessments. Lewis Publishers, Boca Raton, LA, pp. 12-24.

Cameron B; Phillips M. 1985. The Residue Kinetics of [Carbon^{14}-Diflubenzuron in the Domestic Pig: Lab Project Number: 4041: 56654/34/85: 131690. Unpublished study prepared by Inveresk Research Corp. 58 p. MRID 42494202.

Cameron B; Dunsire J; Gifford L; et al. 1989. The Disposition of Carbon^{14}-Diflubenzuron in the Lactating Goat: Lab Project Number: 138261: 4990: 56654/02/90. Unpublished study prepa- red by Invaresk Research International. 63 p. MRID 42060901.

Cameron B; Henderson A; McGuire G. 1990. The Metabolism of [Carbon^{14} Diflubenzuron in the Rat: Profiling of Radioactivity in Urine, Faeces and Bile: Lab Project Number: 139768: 56629/64/ 90: 6255. Unpublished study prepared by Inveresk Research International. 74 p. MRID 41919001.

Cannon GE; Krize JW. 1975. TH 6040 Egg to Egg Reproduction Study in Fathead Minnows Treated at .1, .05, .025, .0125, to .00675 PPM: Laboratory No. 5E 6094. (Unpublished study received Feb 10, 1976 under 6G1744; prepared by Cannon Laboratories, Inc., submitted by Thompson-Hayward Chemical Co., Kansas City, Kans.; CDL:094974-I). MRID 00038616.

Cannon G; Krize J. 1976. TH-6040 Egg to Egg Reproduction Study in Fathead Minnows: Laboratory No. 5E-6094. (Unpublished study received Jul 19, 1976 under 148-1262; prepared by Cannon Laboratories, Inc., submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL:096209-I; 225306). MRID 00099755.

Cardona R. 1999. Evaluation of the Agency's Rationale for Incorporation of PCA (para-chloroaniline) and CPU. (4-chlorophenyl urea) in the EPA Risk Assessment for Diflubenzuron. Unpublished study prepared by Uniroyal Chemical Co., Inc. 10 p. MRID 44871301.

Cardona R. 2001. Evaluation of the Potential Toxicity of CPU(4-chlorophenylurea) a Plant Metabolite of Diflubenzuron. Unpublished study prepared by Uniroyal Chemical Company. 4 p. MRID 45421601.

Catangui MA; Fuller BW; Walz AW; Boetel MA; Brinkman MA. 1996. Abundance, diversity, and spatial distribution of ants (Hymenoptera: formicidae) on mixed-grass rangelands treated with diflubenzuron. Environ Entomol. 25: 757-766.

Cecil H; Miller R; Corley C. 1981. Feeding three insect growth regulators to White Leghorn Hens: Residues in eggs and tissues and effects on production and reproduction. Poultry Science 60:2017-2027. MRID 00156781.

Centa AC; Zucchinetti G; Di Pietro P. 1985. Methemoglobinemia in the newborn and nursing infant: Genetic and acquired forms. Pathologica 77(1052): 659-665. Cited in USDA 1995.

Chandel RS; Gupta PR. 1992. Toxicity of diflubenzuron and penfluron to immature stages of *Apis cerana* indica F and *Apis mellifera* L. Apidologie. 23(5): 465-473 Cited in USDA 1995.

Chapman RA; Tu CM; Harris CR; Harris C. 1985. Persistence of diflubenzuron and Bay Sir 8514 in natural and sterile sandy loam and organic soils. Journal of Environmental Science and Health B20(5):489-497; 1985.

Chesterman H; Heywood R; Barker MH; et al. 1974. Du 112307: Toxicity in Repeated Dietary Administration to Beagle Dogs (Repeated Administration for 13 Weeks): PDR169/74157. (Unpublished study received Feb 10, 1976 under 6G1744; prepared by Huntingdon Research Centre, submitted by Thompson-Hayward Chemical Co., Kansas City, Kans.; CDL:094963-G). MRID 00038706.

Christiansen E. 1987. Effects of a chitin synthesis inhibitor insecticide on crab larvae. Invest Pesq. 51(Suppl 1): 526-527.

Chung K; Starrett S; Chung Y; Ro KS. 1998. Pesticides and herbicides. Water Environment Research. 70(4): 693-697.

Clements RG; Nabholz JV; Zeeman M. 1996. Estimating Toxicity of Industrial Chemicals to Aquatic Organisms Using Structure-activity Relationships. Available at: http://www.epa.gov/oppt/newchems/sarman.pdf.

Cochran M; Poling P. 1995. Chapter 9. Pollinating Insects – Honey Bees. pp. 77-80 in: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Colley J; Offer J. 1977. Effects of Du 112307 in Dietary Administration to Rats for 104 Weeks: Revaluated Pathological Data: PDR171/75945. (Unpublished study received Feb 6, 1978 under 148-1259; prepared by Huntingdon Research Centre, England, submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL:096787-B). MRID 00099712.

Colley J; Batham P; Heywood R; et al. 1981. The Effects of Dietary Administration of Diflubenzuron to Male and Female HC/CFLP Mice for 14 Weeks: Volume 1: HRC Report No. PDR/294/ 80185. Final rept. (Unpublished study received Aug 10, 1981 under 148-1268; prepared by Huntingdon Research Centre, Eng., submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL:245651-A; 245652). MRID 00114330.

Colley J; Heywood R; Street A. 1984. The Effect of Diflubenzuron Given by Oral Administration with the Feed on Toxicity and Tumour Development in Male and Female HC/CFLP Mice: Final Report: PDR 360/831096/B. Unpublished study prepared by Huntingdon Research Centre. 2716 p. MRID 00142490.

Colwell A; Schaefer C. 1980. Diets of *Ictalurus nebulosus* and *Pomoxis nigromaculatus* altered by diflubenzuron. Can J Fish Aquat Sci. 377(4): 632-639. MRID 00160063.

Cook J. 2004. Joseph L. Cook, Supervisory Entomologist, Gypsy Moth EIS Project Leader. 180 Canfield Street, Morgantown, WV 26505. Personal communication to Patrick Durkin, SERA Inc., concerning the use of buffer zones in the application of diflubenzuron, Jan 5, 2004.

Cooper RJ; Dodge KM; Martinat PJ; Donahoe SB; Whitmore RC. 1990. Effect of diflubenzuron application on eastern deciduous forest birds. J Wildl Manage. 54: 486-493.

Coppen GDA; Jepson PC. 1996. Comparative laboratory evaluation of the acute and chronic toxicology of diflubenzuron, hexaflumuron and teflubenzuron against II instar desert locust, (*Schistocerca gregaria*) (Orthoptera: acrididae). Pestic Sci. 46: 183-190.

Coppen GDA; Jepson PC. 1996. The effects of the duration of exposure on the toxicity of diflubenzuron, hexaflumuron and teflubenzuron to various stages of II instar *Schistocerca gregaria*. Pestic Sci. 46: 191-197.

Cunningham PA. 1975. Effects of Dimilin (TH-6040) on Reproduction in the Brine Shrimp, *Artemia salina*. (Unpublished study received Dec 23, 1976 under 148-1258; prepared by North Carolina State Univ., Dept. of Genetics, submitted by Thompson- Hayward Chemical Co., Kansas City, Kans.; CDL:095663-L). MRID 00073933.

Cunningham PA. 1986. A review of toxicity testing and degradation studies used to predict the effects of diflubenzuron Dimilin on estuarine crustaceans. Environ Pollut Ser A Ecol Biol. 40 (1): 63-86.

Cunningham PA; Myers LE. 1987. Effects of diflubenzuron Dimilin on survival molting and behavior of juvenile fiddler crabs, *Uca pugilator*. Arch Environ Contam Toxicol. 16 (6): 745-752.

De Clercq D; Vinuela E; Smagghe G; Degheele D. 1995a. Transport and kinetics of diflubenzuron and pyriproxyfen in the beet armyworm, *Spodoptera exigua*, and its predator *Podisus maculiventris*. Entomologia Experimentalis et Applicata. 76 (2): 189-194.

De Clercq P; De Cock A; Tirry L; Vinuela E; Degheele D. 1995b. Toxicity of diflubenzuron and pyriproxyfen to the predatory bug *Podisus maculiventris*. Entomologia Experimentalis et Applicata. 74(1): 17-22.

De Sapio R. 1976. Calculus for the Life Sciences. W.H. Freeman and Company, San Francisco, California. 740 pp.

De Sousa G; Fontaine F; Pralavorio M; Botta-Fridlund D; Letreut Y; Rahmani R. 1997. Insecticide cytotoxicity and Cyp1a1/2 induction in primary human and rat hepatocyte cultures. Toxicology in Vitro. 11(5): 451-457.

Deakle JP; Bradley JR. 1982. Effects of early season applications of diflubenzuron and azinphosmethyl on population levels of certain arthropods in cottonfields. Journal of the Georgia Entomological Society 17(2):200-204. Cited in USDA 1995.

Deka MK; Hazarika LK. 1995. Aberrant oviposition behaviour of rice hispa, *Dicladispa armigera* (Coleoptera: chrysomelidae) influenced by diflubenzuron. Crop Research (Hisar). 10(1): 74-79.

Delbeke F; Vercruysse P; Tirry L; De Clercq P; Degheele D. 1997. Toxicity of diflubenzuron, pyriproxyfen, imidacloprid and diafenthiuron to the predatory bug *Orius laevigatus* (Het.: anthocoridae). Entomophaga. 42: 349-358.

Delescluse C; Ledirac N; De Sousa G; Pralavorio M; Lesca P; Rahmani R. 1998. Cytotoxic effects and induction of Cytochromes P-450 1a1/2 by insecticides, in hepatic rr epidermal cells: binding capability to the Ah receptor. Toxicology Letters (Shannon). 96-97(Spec. Issue): 33-39.

Demedts P; Wauters A; Watelle M; Neels H. 1997. Pitfalls in discriminating sulfhemoglobin from methemoglobin. Clin Chem. 43(6 Pt 1):1098-9. Available at: http://www.clinchem.org/cgi/content/full/43/6/1098

Demilo AB; Gelman DB; Bordas B. 1997. Benzoylbiuret insect chitin inhibitors: structure-activity correlations derived from an in vitro clasper assay and an in vivo mosquito adult emergency assay. Journal of Entomological Science. 32(2): 212-228.

De Reed RH. 1982. A field study on the possible impact of the insecticide diflubezuron on insectivorous birds. Agro-Ecosystems. 7: 327-342.

Dowdy DL; Mckone TE. 1997. Predicting plant uptake of organic chemicals from soil or air using octanol/water and octanol/air partition ratios and a molecular connectivity index. Environmental Toxicology and Chemistry; 16(12): 2448-2456.

Downey D. 1995. Chapter 17. Breakdown Products of Diflubenzuron. pp. 161-167 in: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Drozdick M. 1998a. Theoretical Discussion of Formation of Impurities for Dimilin 4L: Lab Project Number: 95163. Unpublished study prepared by Uniroyal Chemical Co., Inc. 4 p. {OPPTS 830.1670}. MRID 44574303.

Drozdick M. 1998b. Product Identity and Composition for Dimilin 2L: Lab Project Number: 98051. Unpublished study prepared by Uniroyal Chemical Co., Inc. 4 p. {830.1550}. MRID 44574501.

Drozdick M. 1998c. Theoretical Discussion of Formation of Impurities for Dimilin 2L: Lab Project Number: 98053. Unpublished study prepared by Uniroyal Chemical Co., Inc. 4p. {830.1670}. MRID 44574503.

Drozdick M. 1998d. Product Identity and Composition for Dimilin 25W: Lab Project Number: 95195. Unpublished study prepared by Uniroyal Chemical Co., Inc. 4 p. {830.1550}. MRID 44574901.

Drozdick M. 1998e. Theoretical Discussion of Formation of Impurities for Dimilin 25W: Lab Project Number: 95197. Unpublished study prepared by Uniroyal Chemical Co., Inc. 4 p. {830.1670}. MRID 44574902.

Dubey T. 1995. Chapter 5. Aquatic Fungi. pp. 31-40 in: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Dubois M; Plaisance H; Thome J-P; Kremers P. 1996. Hierarchical cluster analysis of environmental pollutants through P-450 induction in cultured hepatic cells: indications for a toxicity screening test. Ecotoxicology and Environmental Safety. 34(3): 205-215.

Dunsire J; Cameron B; Speirs G. 1990. The Disposition of Carbon[14] Diflubenzuron in the Rat: Lab Project Number: 13919- 7: 4924: 56654/13/90. Unpublished study prepared by Inveresk Research International. 82 p. MRID 41720901.

Durkin PR. 1994. Comparison and Summary of Human Health Risk Assessments for the USDA Control and Eradication Programs. In Proceedings of the 1994 Annual Gypsy Moth Review, D.H. Hilburn, K.J.R. Johnson, and A.D. Mudge (eds), U.S. Department of Agriculture, Salem, Oregon, pp. 170-182.

Durkin PR; Diamond G. 2002. Neurotoxicity, Immunotoxicity, and Endocrine Disruption with Specific Commentary on Glyphosate, Triclopyr, and Hexazinone. Final Report. SERA TR 01-43-08-04a dated January 30, 2002. Available at www.fs.fed.us/foresthealth/pesticide/risk.htm.

Durkin PR; Rubin L; Withey J; Meylan W. 1995. Methods of assessing dermal absorption with emphasis on uptake from contaminated vegetation. Toxicol. Indust. Health. 11(1): 63-79. .

Dzialo D; Maynard P. 1999. Aerobic Aquatic Metabolism of (Carbon-14)-Diflubenzuron: Lab Project Number: 97011: 97012: F97317-811. Unpublished study prepared by Uniroyal Chemical Company and Springborn Laboratories. 652 p. MRID 44895001.

Ecobichon DJ. 1998. Occupational Hazards of Pesticide Exposure – Sampling, Monitoring, Measuring. Taylor & Francis, Philadelphia, PA. 251 pp.

Edwards PJ. 1995. Chapter 2. Site Characteristics. pp. 6-13 in: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Eisenbeis G; Lenz R; Heiber T. 1999. Organic residue decomposition: the minicontainer-system a multifunctional tool in decomposition studies. Environmental Science and Pollution Research International. 6(4): 220-224.

Eisler R. 1992. Diflubenzuron hazards to fish, wildlife, and invertebrates: a synoptic review. Biological Report No. 4. Contaminant hazard review No. 25. U.S. Fish and Wildlife Service. Available from : NTIS/PB94-120136.

Elliot RH; Iyer R. 1982. Toxicity of diflubenzuron to nymphs of the migratory grasshopper *Melanoplus sanguinipes* (Orthoptera: Acrididae). Canadian Entomologist 114(6):479-484. Cited in USDA 1995.

El-Wakil HB; Attia AM. 1999. Effect of selected insecticides on terrestrial snails *Eobania vermiculata* (Muller) and *Theba pisana* (Muller) with respect to some morphological changes in Egypt. J Environ Sci Health, Part B, Pestic Food Contam Agric Wastes. 1: 47-60.

Emmett, B.J.; Archer, B.M. 1980. The toxicity of diflubenzuron to honey bee (*Apis mellifera* L.) colonies in apple orchards. Plant Pathology 29(4):177-183. Cited in USDA 1995.

Everts, J.W. 1990. Environmental effects of chemical locust and grasshopper control, a pilot study. Project Report. ECLO/SEN/003/NET, Food and Agriculture Organization of the UN, Rome. Project Report to FAO. 277 pp. Cited in USDA 1995.

ExToxNet. 1993. Extension Toxicology Network: A Pesticide Information Project of Cooperative Extension Offices of Cornell University, Michigan State University, Oregon State University, and University of California at Davis. Online. Report Dated 9/93.

Farlow J. 1976. Ecological Impact of Dimilin on the Aquatic Founa of a Louisiana Coastal Marsh. Doctoral dissertation, Louisiana State Univ. and Agricultural and Mechanical College, Dept. of Entomology. (Unpublished study received Dec 23, 1976 under 148-1258; submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL:095650-K). MRID 00099678.

Farlow JE; Breaud TP; Steelman CD; Schilling PE. 1978. Effects of the insect growth regulator diflubenzuron on non-target aquatic populations in a Louisiana intermediate marsh. Environ Entomol. 7(2): 199-204.

Ferioli A; Barbieri F. 1994. Health surveillance of pesticide workers a manual for occupational health professionals substituted ureas. Toxicology. 91(1): 63-69.

Fink R. 1973. Final Report: Eight-Day Dietary LD_{50}--Bobwhite Quail: Project No. 553-117. (Unpublished study received Apr 7, 1976 under 148-1259; prepared by Environmental Sciences Corp., submitted by Thompson-Hayward Chemical Co., Kansas City, Kans.; CDL:096227-F). MRID 00039080.

Fink R; Petrocelli SR. 1973. Final Report: Eight-Day Dietary LD_{50}--Mallard Ducks: Project No. 553-118. (Unpublished study received Feb 10, 1976 under 6G1744; prepared by Environmental Sciences Corp., submitted by Thompson-Hayward Chemical Co., Kansas City, Kans.; CDL:094974-E). MRID 00038613.

FIRST, 2001. FQPA Index Reservoir Screening Tool, Version 1.0 August 1, 2001, Environmental Fate and Effects Division, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C. Available at: http://www.epa.gov/oppefed1/models/water/first_description.htm.

Fischer SA; Hall LW. 1992. Environmental concentrations and aquatic toxicity data on diflubenzuron (Dimilin). Crit Rev Toxicol. 22: 45-79.

Fletcher JS; Nellessen JE; Pfleeger TG. 1994. Literature review and evaluation of the EPA food-chain (Kenega) nomogram, an instrument for estimating pesticide residues on plants. Environ Toxicol Chem. 13(9):1383-1391.

Friedlander B. 1999. The Odor of Diflubenzuron Technical: Final Report: Lab Project Number: 99003: GRL-11250. Unpublished study prepared by Uniroyal Chemical Co. 17 p. {OPPTS 830.6304}. MRID 44774202.

Gamon M; Saez A; Pelegri R; Peris I; Cuadra Jg De la; Coscolla R. 1998. Liquid chromatographic determination of five benzoylurea insecticides in fruit and vegetables. J AOAC Int. 81: 1037-1042.

Garces T; Colavita J. 1985. Residues of Diflubenzuron in Milk Following the Feeding of a Diflubenzuron 0.77% Premix-concentrate Mixture to Lactating Dairy Cows for 28 Consecutive Days. Unpublished study prepared by American Cyanamid Co. 69 p. MRID 00155420.

Gay M; Wang R; Long S. 1999. Metabolism of (U-(carbon-14)-Phenyl)-4-Chlorophenylurea by Male Fisher (sic) Rats: Lab Project Number: 98203P. Unpublished study prepared by Uniroyal Chemical Co., Inc. 133 p. {OPPTS 870.7485} MRID 44875501.

GENEEC, 2001. Generic Estimated Environmental Concentrations, Version 2.0. August 1, 2001. Environmental Fate and Effects Division, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C. Available at: http://www.epa.gov/oppefed1/models/water/geneec2_description.htm.

Gijswijt MJ. 1978. Investigations with Dimilin on bees. Crop Protection Division, Phillips-Duphar B.V., Graveland, the Netherlands. Cited in USDA 1995.

Goldenthal E. 1996. 21-Day Dermal Toxicity Study in Rats: Dimilin Technical (Diflubenzuron). Lab Project Number: 399-186. Unpublished study prepared by MPI Research. 164 p. MRID 43954101.

Goldenthal E. 1999. 7-Day Dietary Toxicity Study in Rats: (para-chloroaniline and para-chlorophenyl urea):. Lab Project Number: 399-203. Unpublished study prepared by MPI Research. 125 p MRID 44871302.

Goldenthal E. 1999. Determination of Methemoglobin Formation After a Single Oral Gavage Dosing of p-chloroaniline and p-chlorophenylurea in Rats: Lab Project Number: 399-204. Unpublished study prepared by MPI Research. 40 p. MRID 44871303.

Gordon R; Cornect M. 1986. Toxicity of the insect growth regulator diflubenzuron to the rove beetle *Aleochara-bilineata,* a parasitoid and predator of the cabbage maggot *Delia-radicum.* Entomol Exp Appl. 42(2): 179-186.

Granett J; Dunbar D M. 1974. TH 6040: Laboratory and field trials for control of gyspy moths. Journal of Economic Entomology 68(1):99-102. Cited in USDA 1995.

Granett J; Hejazi M J. 1983. Synergism of 2 benzoylphenyl urea insect growth regulators. Journal of conomic Entomology 76(3):403-406. Cited in USDA 1995.

Granett .; Weseloh RM.1977. Dimilin toxicity to the gypsy moth larval parasitoid, *Apanteles melanoscelus.* Journal of Economic Entomology 68(5): 577-580. Cited in USDA 1995.

Granett J; Dunbar DM; Weseloh RM. 1976. Gypsy moth control with Dimilin sprays timed to minimize effects on the parasite *Apanteles melanoscelus.* Journal of Economic Entomology 69(3):403-404. Cited in USDA 1995.

Granett J; Bisabri-Ershadi B; Hejazi M J. 1982. Some parameters of benzoylphenyl ureas toxicity to beet armyworms *Spodoptera exigua* Lepidoptera Noctuidae. Journal of conomic Entomology 76(3): 399-402. Cited in USDA 1995.

Greenough R; Goburdhun R; Hudson P et al. 1985. Diflubenzuron 52 Week Oral Toxicity Study in Dogs: Project No. 630146. Unpublished study prepared by Inveresk Research International. 353 p. [Basis for RfD] MRID 00146174.

Griffith MB; Barrows EM; Perry SA. 1996. Effects of aerial application of diflubenzuron on emergence and flight of adult aquatic insects. J Econ Entomol. 89: 442-446.

Griffith MB; Barrows EM; Perry SA. 2000. Effect of diflubenzuron on flight of adult aquatic insects (Plecoptera, trichoptera) following emergence during the second year after aerial application. J Econ Entomol. 93: 1695-1700.

Gulka G; Doscher CM; Watabe N. 1980. Toxicity and molt-accelerating effects of diflubenzuron on the barnacle, *Balanus eburneus.* Bull Environ Contam Toxicol. 25(3): 477-481.

Gundrum P; Iskra A; Wimmer MJ. 1995. Chapter 13. Terrestrial Fungi in Leaf Litter. pp. 121-129 in: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Hagler L; Askew EW; Neville JR; Mellick RW; Coppes RI; Lowder JF. 1981. Influence of dietary iron deficiency on hemoglobin, myoglobin, their respective reductases, and skeletal

muscle mitochondrial respiration. American Journal of Clinical Nutrition 34(10): 2169-2177. Cited in USDA 1995.

Hall D. 1986. Toxicity of Two Insect Growth Regulators on *Ceriodaphnia dubia*. Unpublished study prepared by Colorado State University, Department of Fishery & Wildlife. 168 p. MRID 40130601.

Hanratty MP; Liber K. 1996. Evaluation of model predictions of the persistence and ecological effects of diflubenzuron in a littoral ecosystem. Ecological Modeling. 90(1): 79-95.

Hansen S; Garton R. 1982a. Ability of standard toxicity tests to predict the effects of the insecticide diflubenzuron on laboratory stream communities. Can J Fish Aquat Sci. 39: 1273-1288 {Also submitted to U.S. EPA/OPP as MRID 00156553}.

Hansen S; Garton R. 1982b. The effects of diflubenzuron on a complex laboratory stream community. Archives of Environmental Contamination and Toxicology. 11: 1-10 {Also submitted to U.S. EPA/OPP as MRID 00156582}.

Harned W; Relyea D. 1997. Computer Modeling of the Fate of Diflubenzuron in Pond Water Resulting from Treatment of Citrus in Florida. (Revised 09/26/97): Lab Project Number: 96108. Unpublished study prepared by Uniroyal Chemical Co., Inc. 119 p. MRID 44460703.

Harrahy EA; Wimmer MJ; Perry SA; Faber DC; Miracle JE; Perry WB. 1993. Persistence of diflubenzuron on Appalachian forest leaves in stream water. J Agric Food Chem. 41: 2191-2196.

Harrahy EA; Perry SA; Wimmer MJ; Perry WB. 1994. The effects of diflubenzuron (Dimilin) on selected mayflies (Heptageniidae) and stoneflies (*Peltoperlidae* and *Pteronarcyidae*). Environ Toxicol Chem. 13: 517-522.

Hastings F; Zhong H. 1995. Chapter 15. Aquatic Environment. pp. 141-145 in: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Heinrichs, E.A.; Gastal, H.A. de O.; Galileo, M.H.M. 1979. Incidence of natural control agents of the velvetbean caterpillar and response of its predators to insecticide treatments in Brazilian soybean fields. Pesquisa Agropecuaria Brasileira 14(1):79-88. Cited in USDA 1995.

Hendersen, C.; Smith, H.D.; Jorgensen, C.D. 1977. Small mammal responses to experimental pesticide applications in coniferous forests. Final report Douglas-fir tussock moth R&D Program RA-8, Pacific Southwest Forest and Range Experiment Station, Berkeley. 95 pp. Summarized in USDA 1995.

Hester P; Olson M; Floore T. 1986. Effects of diflubenzuron on three estuarine decapods, *Callinectes* sp., *Palaemonetes pugio* and *Uca pugilator*. Journal of the Florida Anti-Mosquito Association. 28: 8-14 {Also submitted to EPA as MRID 40093602}.

Hobson JF. 2001. Ecological Risk Assessment of Diflubenzuron Use in Forestry Applications. MoringStar Consulting, Inc. 20 pages. Appended to Schocken et al. 2001, MRID 45517001.

Hoffman G. 1997. An Acute (4-hour) Inhalation Toxicity Study of Dimilin 2L in the Rat via Nose-Only Exposure: Final Report: Lab Project Number: 96-5300. Unpublished study prepared by Huntingdon Life Sciences. 93 p. MRID 44574506.

Hoffman RS; Sauter D. 1989. Methemoglobinemia resulting from smoke inhalation. Veterinary and Human Toxicology. 31(2): 168-170. Cited in USDA 1995.

Horst MN; Walker AN. 1995. Biochemical effects on diflubenzuron on chitin synthesis in the postmolt blue crab callinectes sapidus. Journal of Crustacean Biology. 15(3): 401-408.

House, V.S.; Ables, J.R.; Morrison, R.K.; Bull, D.L. 1980. Effect of diflubenzuron formulations on the egg parasite *Trichogramma pretiosum*. Southwestern Entomologist 5(2):133-138. Cited in USDA 1995.

Hugla JL; Goffinet G; Kremers P; Dubois M; Lambert V; Stouvenakers N; Thome JP. 1996. Ultrastructural modifications in cultured fetal quail hepatocytes exposed to pesticides and PCBs. Ecotoxicology and Environmental Safety. 34(2): 145-155.

Hurd MK; Perry SA; Perry WB. 1996. Nontarget effects of a test application of diflubenzuron to the forest canopy on stream macroinvertebrates. Environ Toxicol Chem. 15: 1344-1351.

Hutchinson TH; Solbe J; Kloepper-Sams PJ. 1998. Analysis of the Ecetoc Aquatic Toxicity (EAT) database. III - Comparative toxicity of chemical substances to different life stages of aquatic organisms. Chemosphere. 36: 129-142.

IARC (International Agency for Research on Cancer (IARC). 1999. p-Chloroaniline: Summary of data reported and evaluation. Available at: http://www.iarc.fr/
Last Updated August 22, 1997.

Iskra A; Binion D; Gundrum P; Stephenson SL. 1995. Chapter 12. Decomposer and Ectomycorrhizal Fungi. pp. 106-120 in: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Ivie GW. 1978. Fate of diflubenzuron in cattle and sheep. J. Agric. Food Chem. 26(1): 81-89.

Jackson G. 1976. Dimilin (TH6040): Biologic Impact on Pond Organisms. (Unpublished study received Jul 31, 1978 under 148-1259; prepared in cooperation with U.S. Fish and Wildlife Service, Southeastern Fish Cultural Laboratory, submitted by Thompson- Hayward Chemical Co., Kansas City, KS; CDL:234511-W). MRID 00099891.

Jech, L.E.; Foster, R.N.; Colletto, D.; Walgenbach, D.D.; Roland, T.J.; Rodriguez, G.D.; Bohls, R.; Houston, R.D.; Meeks, W.K.; Queener, R.L.; Jackson, C.L.; Dines, J.L.; Puclik, M.J.; Scott, A.K. 1993. Field evaluation of diflubenzuron and carbaryl bran baits against grasshopper (Orthoptera: Acrididae) populations in South Dakota. Journal of Economic Entomology 86:557-565. Cited in USDA 1995.

Jenkins, V.K.; Perry, R.R.; Ahmed, A.E.; Ives, K. 1986. Role of metabolism in effects of diflubenzuron on growth of B16 melanomas in mice. Investigational New Drugs 4: 325-335.

Jepson PC; Yemane A. 1991. Toxicity of diflubenzuron to nymphs of the desert locust *Schistocerca gregaria* Forsk. (Orthoptera: Acrididae). Pesticide Science 34(1):92-93. Cited in USDA 1995.

Johnson W; Finley MR. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. USDI Publication 137, Washington DC. Cited in U.S. EPA 1997a.

Julin AM; Sanders HO. 1978. Toxicity of the IGR, diflubenzuron, to freshwater invertebrates and fishes. Mosquito News 38(2):256-259. Cited in USDA 1995.

Kadam NV; Dalvi CS; Dumbre RB. 1995. Efficacy of diflubenzuron a chitin synthesis inhibitor against diamondback moth. Journal of Maharashtra Agricultural Universities. 20(1): 17-20.

Kalafatic M. 1997. Regeneration and asexual reproduction of *Hydra oligactis* treated with different pesticides. Biologia (Bratislava). 52(3): 475-480.

Kalafatic M; Kopjar N. 1994. Response of green hydra to the treatment with different pesticides under laboratory conditions. Zeitschrift Fuer Angewandte Zoologie. 80(2): 213-223.

Kavanagh P. 1988. Diflubenzuron: Oral (Gavage) Rat Teratology Limit Study: Lab Project Number: PHD/11/87: 56645/68/87. Unpublished study prepared by Toxicol Laboratories Ltd. 91 p. MRID 41703504. Summarized in U.S. EPA 1997a.

Kavanagh P. 1988b. Diflubenzuron: Oral (Gavage) Rabbit Teratology Limit Study: Lab Project Number: PHD/12/87: 56645/79/87. Unpublished study prepared by Toxicol Laboratories Ltd. 78 p. MRID 41703505. Summarized in U.S. EPA 1997a.

Keet C. 1983. Review of relevant toxicity data of diflubenzuron and its formulation, Dimilin WP-25, in relation to exposure of field personnel. Duphar B.V., Weesp, the Netherlands: Duphar Report No. 56645/35/83; April 1983; 13 p.

Keet C. 1984a. Summary of the report: oncogenicity study in rats, diflubenzuron. Project No. 553-122. Duphar B.V. Weesp, the Netherlands: Duphar Report No. 56645/48/84; 2 April 1984; 12 p. + app.

Keet C. 1984b. Summary of the report: the effect of diflubenzuron given by oral administration with the feed on toxicity and tumor development in male and female hc/cflp mice. PRD 360/83. Duphar B.V. Weesp, the Netherlands: Duphar Report No. 56645/49/84; 15 May 1984; 12 p. + app.

Keet, C.; Kemp, A.; Mass, W. 1982. Effects of diflubenzuron on methaemoglbin and sulphhaemoglobin levels and other red blood cell related parameters in mice, rats, cats, dogs and sheep. Duphar Report DI-No. 4770; presented at the International Conference on Environmental Hazards of Agrochemicals in Developing Countries; November 8-12; Alexandria, Egypt. Unpublished report prepared by Duphar B.V., Crop Protection Division, the Netherlands; 7 p.

Keever DW; Bradley Jr Jr; Ganyard MC. 1977. Effects of diflubenzuron (Dimilin) on selected beneficial arthropods in cotton fields. Environ. Entomol. 6(5): 732-736.

Keller L. 1997. A Comparison of the Effects of Diflubenzuron (Dimilin) and Tebufenozide(Confirm/Mimic) on Nontarget Terrestrial and Aquatic Arthropods and Aquatic Community Structure. Lab Project Number: 96R-1113. Unpublished study prepared by Rohm and Haas Co. 32 p. MRID 44221902.

Key BD; Howell RD; Criddle CS. 1997. Fluorinated organics in the biosphere. Environmental Science and Technology. 31(9): 2445-2454.

Khakoo GA; Maconochie IK; Jaffe P. 1993. An unusual blue baby. Journal of Royal Society of Medicine (London) 86(12): 730-731. Cited in USDA 1995.

Khebbeb MEH; Delachambre J; Soltani N. 1997. Lipid metabolism during the sexual maturation of the mealworm (*Tenebrio molitor*): effect of ingested diflubenzuron. Pestic Biochem Physiol. 58: 209-217.

Khoo BK; Forgash AJ; Respicio NC; Ramaswamy SB. 1985. Multiple progeny production by gypsy moth parasites *Brachymeria*-spp hymenoptera chalcididae following exposure to diflubenzuron. Environ Entomol. 14(6): 820-825.

Knuth ML; Heinis LJ. 1995. Distribution and persistence of diflubenzuron within littoral enclosure mesocosms. J Agric Food Chem. 43: 1087-1097.

Knutson RD; Smith EG. 1999. Impacts of Eliminating Organophosphates and Carbamates From Crop Production. Agricultural and Food Policy Center, Texas Agricultural Extension Service, Texas A&M University. Report dated April 1999. Available at: http://afpc1.tamu.edu/pesticides.htm.

Koopman TSM. 1977. Acute Oral Toxicity Study with Du 112307 W.P. 25% in Mice and Rats: Report No. 56645/3/77. (Unpublished study received Jun 22, 1977 under 6F1773; prepared by Philips-Duphar, B.V., Netherlands, submitted by Thompson- Hayward Chemical Co., Kansas City, Kans.; CDL:096166-L) Philips-Duphar BV, Department of Toxicology. MRID 00070025.

Korpalski S. 1996. Dimilin 25W and 2L on Cotton: Dislodgeable Foliar Residue Study: Lab Project Number: RP-95035: CEJ-95-005: AWD-95-911. Unpublished study prepared by Excel Research Services, Inc.; Coastal Ag Research, Inc.; and Colorado Analytical Research & Development. MRID 44081401.

Korpalski S. 1996. Micromite-25W on Oranges: Dislodgeable Foliar Residue Study: Lab Project Number: RP-95034: KHG-95-201: KHG-95-202. Unpublished study prepared by Entocon, Inc.; Agvise, Inc.; and Colorado Analytical Research & Development Corp. 345 p. MRID 44081402.

Kotze AC; Sales N; Barchia IM. 1997. Diflubenzuron tolerance associated with monooxygenase activity in field strain larvae of the Australian sheep blowfly (Diptera: calliphoridae). J Econ Entomol. 90: 15-20.

Koyanagi T; Morita M; Fujii Y. 1998. Synthesis and insecticidal activity of alkylated n-benzoyl-n'-phenylureas and their toxicity to aquatic invertebrate. Journal of Pesticide Science. 23: 250-254.

Kühn R; Pattard M; Pernak KD; Winter A. 1989a. Results of the harmful effects of selected water pollutants (anilines, phenols, aliphatic compounds) to Daphnia magna. Water Research, 23:495-499. Cited in WHO 2003.

Kühn R; Pattard M; Pernak KD; Winter A. 1989b. Results of the harmful effects of water pollutants to Daphnia magna in the 21 day reproduction test. Water Research, 23:501-510.

Kurczewski F; Wang C; Grimble D; Smith R; Brezner J. 1975. Environmental impact of dimilin. A final report: Effects of dimilin upon microorganisms in leaf litter and forest soil. Syracuse, New York, State University of New York, pp 28-43. Summarized in WHO 1996.

Kreutzweiser D; England L; Shepherd J; Conklin J; Holmes S. 2001. Comparative effects of a genetically engineered insect virus and a growth-regulating insecticide on microbial communities in aquatic microcosms. Ecotoxicol Environ Safety. 48(1):85-98.

Kubena LF. 1981. The influence of diflubenzuron on several weight characteristics in growing male broiler and layer chickens. Poultry Sci. 60: 1175-1182.

Kubena, L.F. 1982. The influence of diflubenzuron on several reproductive characteristics in male and female layer-breed chickens. Poultry Science 61: 268-271.

Kubena, L.F.; Witzel, D.A. 1980. Nutritional and metabolic aspects of toxicity in livestock and poultry. Toxicology Research Projects Directory 5(10): 268-271.

Kuijpers L. 1988. The Acute Toxicity of Diflubenzuron to Daphnia magna: Laboratory Project ID: C.303.51.008. Unpublished study prepared by Duphar B.V. 16 p.   MRID 40840502.

Kuijpers L. 1989. The side-effects of diflubenzuron (Dimilin) on bees: A review. Philips-Duphar B.V., The Netherlands, Crop Protection Division, Report No. 56635/07/89. Cited in USDA 1995.

Kula C; Rombke J. 1998. Evaluation of soil ecotoxicity tests with functional endpoints for the risks assessment of plant protection products. Environmental Science and Pollution Research International. 5: 55-60.

Kumar S; Dahiya B; Chauhan R; Jaglan MS. 1994. Ovicidal action of diflubenzuron against *Helicoverpa armigera* (Lepidoptera: noctuidae). .

Kumari CVA; Mohamed UVK. 1997. Effect of diflubenzuron treatment on the ovarian carbohydrates of *Spodoptera mauritia* Boisd. Journal of Entomological Research (New Delhi). 21(3): 229-232.

Lahr J. 1998. An ecological assessment of the hazard of right insecticides used in desert locust control, to invertebrates in temporary ponds in the Sahel. Aquatic Ecology. 32(2): 153-162.

Lahr J; Diallo AO; Gadji B; Diouf PS; Bedaux JJM; Badji A; Ndour KB; Andreasen JE; Straalen Nm van. 2000. Ecological effects of experimental insecticide applications on invertebrates in Sahelian temporary ponds. Environ Toxicol Chem. 19: 1278-1289.

Lahr J; Badji A; Marquenie S; Schuiling E; Ndour KB; Diallo AO; Everts JW. 2001. Acute toxicity of locust insecticides to two indigenous invertebrates from Sahelian temporary ponds. Ecotoxicol Environ Safety. 48:66-75.

Landolt JC; Stephenson SL . 1995. Chapter 11. Soil Bacteria and Fungi. pp. 93-105. In: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Laney RF; Hoffman RS. 1992. Methemoglobinemia secondary to automobile exhaust fumes. American Journal of Emergency Medicine 10(5): 426-428. Cited in USDA 1995.

LeBlanc G. 1975. The Chronic Toxicity of Altosids (R), TH-6040, and R-20458 to *Daphnia magna*. (unpublished study received Feb. 8, 1977 under 20954-1; prepared by EG&G, Bionomics, submitted by Zoecon Corp., Palo Alto, CA; CDL:231488-I). MRID 00010865. Cited in U.S. EPA 1997a.

Ledirac N; Delescluse C; Lesca P; Piechocki MP; Hines RN; de Sousa G; Pralavorio M; Rahmani R. 2000. Diflubenzuron, a benzoyl-urea insecticide, is a potent inhibitor of TCDD-induced Cyp1a1 expression in Hepg2 cells. Toxicol Appl Pharmacol. 164: 273-279.

Lee R; Oshima Y. 1998. Effects of selected pesticides metals and organometallics on development of blue crab *Callinectes sapidus* embryos. Marine Environmental Research. 46: 479-482.

Lee BM; Scott GI. 1989. Acute toxicity of temephos, fenoxycarb, diflubenzuron, and methoprene and *Bacillus thuringiensis* var. israelensis to the mummichog (*Fundulus heteroclitus*). Bull Environ Contam Toxicol. 43: 827-832.

Lengen M. 1999. Dimilin Dietary Risk Assessment for para-Chloroaniline (PCA) . Unpublished study prepared by Uniroyal Chemical Co., Inc. 20 p. MRID 44875503.

Liber K; Schmude KL; Corry TD. 1996. Effects of the insect growth regulator diflubenzuron on insect emergence within littoral enclosures. Environ Entomol. 25: 17-24.

Liebhold A; Luzader E; Reardon R; Bullard A; Roberts A; Ravlin W; Delost S; Spears B. 1996. Use of a geographic information system to evaluate regional treatment effects in a gypsy moth (Lepidoptera: lymantriidae) management program. J Econ Entomol. 89: 1192-1203.

Lim, S.-J.; Lee, S.-S. 1982. The toxicity of diflubenzuron to *Oxya japonica* (Willemse) and its effect on molting. Pesticide Science 13:537-544. Cited in USDA 1995.

Livingston R; Koenig C. 1977. Life Cycle Toxicity Tests Concerning the Acute and Chronic Effects of a Pesticide (TH-6040) on the Mummichug (*Fundulus heteroclitus* Walbaum), and Egg-laying Topminnow. (Unpublished study received Feb 6, 1978 under 1481259; prepared by Environmental Planning & Analysis, submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL:096787-M). MRID 00099722.

Lo SC; Agar NC. 1986. NADH-methemoglobin reductase activity in the erythrocytes of newborn and adult mammals. Experientia. 42(11-12): 1264-1265. Cited in USDA 1995.

Lorenz J; Lenz M; Hoffmann KH. 1995. Effects of pH agents on ecdysteroid synthesis in vitro in ovaries and abdominal integument from female adult crickets, *Gryllus bimaculatus* de Geer (Ensifera, gryllidae). Zeitschrift Fuer Naturforschung Section C Biosciences. 50(3-4): 286-293.

Mabury SA; Crosby DG. 1996. Fate and disposition of diflubenzuron in rice fields. Environ Toxicol Chem. 15: 1908-1913.

Madder DJ; Lockhart WL. 1978. A preliminary study of the effects of diflubenzuron and methoprene on rainbow trout (*Salmo gairdneri* Richardson). Bull Environ Contam Toxicol. 20(1): 66-70.

Madder DJ; Lockhart WL. 1980. Studies on the dissipation of diflubenzuron and methoprene from shallow prairie pools. Can. Entomol. 112(2): 173-179.

Madrid, F.J.; Stewart, R.K. 1981. Impact of diflubenzuron spray on gypsy moth parasitoids in the field. Journal of Economic Entomology. 74(1):1-2. Cited in USDA 1995.

Majori G; Romi R; Ali A. 1984. Toxicity of the IGR diflubenzuron to neonate, adult, and gravid female *Daphnia magna* Straus (Cladocera: Daphniidae) in the laboratory. Proceedings and Papers on the 52nd Annual Conference of the California Mosquito & Vector Control Association. 68-70. Cited in USDA 1995.

Mani M; Lakshmi VJ; Krishnamoorthy A. 1997. Side effects of some pesticides on the adult longevity, progeny production and prey consumption of *Cryptolaemus montrouzieri* Mulsant (Coccinellidae, coleoptera). Indian Journal of Plant Protection. 25(1): 48-51.

Marsella AM; Jaskolka M; Mabury SA. 2000. Aqueous solubilities, pH rates and partition coefficients of benzoylphenylurea insecticides. Pest Manag Sci. 56: 789-794.

Marshall VG. 1979. Effects of the insecticide diflubenzuron on soil mites of a dry forest zone in British Columbia, Canada. In: Rodriguez, J.G. ed. Recent Advances in Acarology, vol. 1. New York: Academic Press; 129-134. Cited in USDA 1995.

Marshall, B.L.; Hieb, B.L. (1973) 96-Hour LC50: *Salmo gairdneri*, *Lepomis macrochirus* and *Fundulus heteroclitus*. (Unpublished study received Apr 5, 1974 under 148-1170; prepared by Marine Research Institute, submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL:224671-O) MRID 00056150. Cited in U.S. EPA 1997a.

Martin PJS; Clark JM; Edman JD. 1995. Preliminary study of synergism of acid rain and diflubenzuron. Bull Environ Contam Toxicol. 54: 833-836.

Martinat PJ; Coffman CC; Dodge K; Cooper RJ; Whitmore RC. 1988. Effect of diflubenzuron on the canopy arthropod community in a central Appalachian forest. J Econ Entomol. 81(1): 261-267.

Martinat PJ; Jennings DT; Whitmore RC. 1993. Effects of diflubenzuron on the litter spider and orthopteroid community in a central Appalachian forest infested with gypsy moth (Lepidoptera: lymantriidae). Environ Entomol. 22: 1003-1008.

Martinez-Toledo MV; Gonzalez-Lopez J; De La Rubia T; Moreno J; Ramos-Cormenzana A. 1988. Diflubenzuron and the acetylene-reduction activity of *Azotobacter vinelandii*. SOIL BIOL BIOCHEM; 20 (2). 1988. 225-256.

Mathur GK. 1998. Laboratory evaluation of diflubenzuron: deleterious effects on ovaries of *Poekilocerus pictus* (Fabr.). Entomon. 23(1): 67-68.

Matthenius JC Jr. 1975. Effects of Dimilin on Honey Bees. (New Jersey, Dept. of Agriculture, unpublished study; CDL: 094969-K). (New Jersey, Dept. of Agriculture, unpublished study; CDL: 094969-K). MRID 00038216.

McAlonan W. 1975. Effects of Two Insect Growth Regulators on Some Selected Saltmarsh Non-target Organisms. (Unpublished study received Jul 31, 1978 under 148-1259; submitted by Thomp- son-Hayward Chemical Co., Kansas City, KS; CDL:234511-AA). MRID 00099895.

McCasland CS; Cooper RJ; Barnum DA. 1998. Implications for the use of diflubenzuron to reduce arthropod populations inhabiting evaporation ponds of the San Joaquin Valley, California. Bull Environ Contam Toxicol. 60: 702-708.

McWhorter, R.; Shepard, M., 1977. Response of Mexican bean beetle larvae and the parasitoid Pediobius foveolatus to Dimilin. Florida Entomologist 60:55-56. Cited in USDA 1995.

Mayer FL; Ellersieck MR. 1986. Manual of acute toxicity: interpretation and data base of 410 chemicals and 66 species of freshwater animals. U.S. Department of the Interior, Fish and Wildlife Service, Resource Publication 160, Washington, DC. Cited in USDA 1995.

Mayer RT; Netter KJ; Leising HB; Schachtschabel DO. 1984. Inhibition of the uptake of nucleosides in cultured Harding-Passey melanoma cells by diflubenzuron. Toxicology 30: 1-6.

Medina P; Smagghe G; Budia F; Del Estal P; Tirry L; Vinuela E. 2002. Significance of penetration, excretion, and transovarial uptake to toxicity of three insect growth regulators in predatory lacewing adults. Arch Insect Biochem Physiol. 51(2):91-101.

Medina P; Smagghe G; Budia F; Del Estal P; Tirry L; Vinuela E. 2003. Toxicity and Absorption of Azadirachtin, Diflubenzuron, Pyriproxyfen, and Tebufenozide after Topical

Application in Predatory Larvae of Chrysoperla carnea (Neuroptera: Chrysopidae). Environ. Entomol. 32(1): 196-203.

Medina P; Smagghe G; Budia F; Del Estal P; Tirry L; Vinuela E. 2003. Toxicity and Absorption of Azadirachtin, Diflubenzuron, Pyriproxyfen, and Tebufenozide after Topical Application in Predatory Larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environ. Entomol. 32(1): 196-203.

Meylan, W.; Howard, P. 1998. ECORAR Class Program, Version 0.99d. Syracuse Research Corporation, Syracuse, N.Y. for U.S. Environmental Protection Agency, Office of Pollution, Prevention and Toxics, Washington D.C.

Meylan, W.; Howard, P. 2000. Estimation Program Interface, Version 3.10. Syracuse Research Corporation, Syracuse, N.Y. for U.S. Environmental Protection Agency, Office of Pollution, Prevention and Toxics, Washington D.C.

Miller RW; Cecil HC; Carey AM; Corley C; Kiddy CA. 1979. Effects of feeding diflubenzuron to young male Holstein cattle. Bull. Environ. Contam. Toxicol. 23(4-5): 482-486.

Miller DR; Yendol WE; Ducharme KM; Maczuga S; Reardon RC; Mcmanus MA. 1996. Drift of aerially applied diflubenzuron over an oak forest. Agricultural and Forest Meteorology. 80(2-4): 165-176.

Mineau P; Boersma DC; Collins B. 1994. An analysis of avian reproduction studies submitted for pesticide registration. Ecotoxicology and Environmental Safety. 29(3): 304-329.

Miura T; Takahashi RM. 1974. Insect developmental inhibitors. Effects of candidate mosquito control agents on nontarget aquatic organisms. Environmental Entomology 3:631-636. Cited in USDA 1995.

Moffett M. 1995. Effects, Persistence and Distribution of Diflubenzuron in Littoral Enclosures: Final Report. Unpublished study prepared by U.S. EPA and University of Wisconsin-Superior. 21 p.   MRID 44386201.

Murphy CF; Jepson PC; Croft BA. 1994. Database analysis of the toxicity of antilocust pesticides to non-target, beneficial invertebrates. Crop Protection. 13(6): 413-420.

Murray KF; Christie DL. 1993. Dietary protein intolerance in infants with transient methemoglobinemia and diarrhea. Journal of Pediatrics 122(1): 90-92. Cited in USDA 1995.

Mutanen RM; Siltanen HT; Kuukka VP; Annila EA; Varama MM. 1988. Residues of diflubenzuron and two of its metabolites in a forest ecosystem after control of the pine looper moth *Bupalus piniarius* L. Pestic Sci. 23(2): 131-140.

Nakagawa Y. 1996. Mode of action of benzoylphenylureas. Journal of Pesticide Science. 21(4): 460-467.

Nakagawa Y; Ishii S; Matsumura F. 1996. Diflubenzuron stimulates pH of a 39 kda integumental protein from newly molted American cockroach (*Periplaneta americana*). Insect Biochem Mol Biol. 26: 891-898.

Nation JL; Robinson FA; Yu SJ; Bolten AB. 1986. Influence upon honeybees of chronic exposure to very low levels of selected insecticides in their diet. J APIC Res. 25(3): 170-177.

Neumann, R.; Guyer, W. 1987. Biochemical and Toxicological differences in the Modes of Action of the Benzoylureas. Pesticide Science 20(2):147-156. Cited in USDA 1995.

Newton P. 1999. A 4-Week Inhalation Toxicity Study of Dimilin Technical in Rats: Lab Project Number: 399-205. Unpublished study prepared by MPI Research, Inc. 357 p. MRID 44950601.

Nigg H; Stamper J. 1987. Diflubenzuron Drift into a Pond during Application to Citrus: Project ID: C.303.60.002 and Int. Doc. No. 56637/05/87. Unpublished study prepared by Univ. of Florida, Citrus Research and Education Center. 24 p. MRID 40197002.

Nigg HN; Cannizzaro RD; Stamper JH. 1986. Diflubenzuron surface residues in Florida citrus. Bull Environ Contam Toxicol. 36(6):833-838.

Nilsson A; Engberg G; Henneberg S; Danielson K; De Verdier CH. 1990. Inverse relationship between age-dependent erythrocyte activity of methaemoglobin reductase and prilocaine-induced methemoglobinemia during infancy. British Journal of Anaesthesia (London) 64(1): 72-26. Cited in USDA 1995.

Nimmo DR; Hamaker TL; Moore JC; Sommers CA. 1979. Effect of diflubenzuron on an estuarine crustacean. Bull Environ Contam Toxicol. 22(6): 767-770.

Nimmo W; Willems A; de Wilde P. 1978. Fate of Diflubenzuron Applied to Leaves and Fruits of Apple Trees: Laboratory Project ID: 56635/33/78. Unpublished study prepared by Duphar B.V. 13 p. MRID 40659701.

Nimmo W; De Wilde P; Verloop A. 1984. The degradation of diflubenzuron and its chief metabolites in soils, Part 1: Hydrolytic cleavage of Diflubenzuron. Pestic. Sci. 15: 575-585.

Nimmo WB; Willems A GM; Joustra KD; Verloop A. 1986. The degradation of diflubenzuron and its chief metabolites in soils. Part II fate of 4-chlorophenylurea. Pestic Sci. 17(4): 403-411.

Nimmo WB; Joustra KD; Willems AGM. 1990. The degradation of diflubenzuron and its chief metabolites in soils. Part III. Fate of 2,6-difluorobenzoic acid. Pestic Sci. 29: 39-45.

NRC (National Research Council). 1983. Risk assessment in the Federal government: managing the process. Washington, DC: National Academy Press; 176 p. + app.

O'Connor, T.F.; Moore, R.B. 1975. The effects of Dimilin on small mammals. P.120-139 in The environmental impact of Dimilin (TH 6040) on a forest and aquatic ecosystem. Lake Ontario Environmental Laboratory. LOTEL Report No. 210. Summarized in USDA 1995.

O'Halloran SL; Liber K; Schmude KL; Corry TD. 1996. Effects of diflubenzuron on benthic macroinvertebrates in littoral enclosures. Arch Environ Contam Toxicol. 30: 444-451.

Oberlander H; Silhacek DL. 1998. Mode of action of insect growth regulators in lepidopteran tissue culture. Pestic Sci. 54: 300-302.

Oomen PA; Jobsen JA; Romeijn G; Wiegers GL. 1994. Side-effects of 107 pesticides on the whitefly parasitoid *Encarsia formosa*, studies and evaluated according to EPPO Guideline No. 142. Bulletin OEPP. 24(1): 89-107.

Opdycke J; Menzer R. 1984. Pharmacokinetics of diflubenzuron in two types of chickens. Journal of Toxicology and Environmental Health 12:721-733. MRID 00152501.

PAN Pesticides Database. 2004. Entry for 2,6-Difluorobenzoic acid and 4-chlorophenyl urea. Available at: http://www.pesticideinfo.org/ Search_Ecotoxicity.jsp#Chemicals

Park CG; Yoo JK; Lee JO. 1996. Toxicity of some pesticides to twospotted spider mite (Acari: tetranychidae) and its predator *Amblyseius womersleyi*. Korean Journal of Applied Entomology. 35(3): 232-237.

Pauley TK. 1995a. Chapter 3. Aquatic Salamanders. pp. 14-22 in: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Pauley TK. 1995b. Chapter 6. Aquatic Fungi. pp. 42-52 in: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Paulus R; Roembke J; Ruf A; Beck L. 1999. A comparison of the litterbag-, minicontainer- and bait-lamina-methods in an ecotoxicological field experiment with diflubenzuron and *Btk*. Pedobiologia. 43(2): 120-133.

Perocco, P.; Colacci, A.; Grilli, S. 1993. In vitro cytotoxic and cell transforming activities exerted by the pesticides cyanazine, dithianon, diflubenzuron, procymidone, and vinclozolin on BALB/c 3T3 cells. Environmental and Molecular Mutagenesis 21: 81-86.

Perry SA. 1995a. Chapter 4. Macroinvertebrates. pp. 23-30 in: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Perry WB. 1995. Chapter 10. Invertebrates in Leaf Litter and Soil. pp. 81-92 in: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Perry WB; Christiansen TA; Perry SA. 1997. Response of soil and leaf litter microarthropods to forest application of diflubenzuron. Ecotoxicology. 6(2): 87-99.

Peveling R; Demba SA. 1997. Virulence of the entomopathogenic fungus metarhizium flavoviride gams and rozsypal and toxicity of diflubenzuron, fenitrothion-esfenvalerate and profenofos-cypermethrin to nontarget arthropods in Mauritania. Arch Environ Contam Toxicol. 32: 69-79.

Pichon V; Chen L; Durand N; Le Goffic F; Hennion M-C. 1996. Selective trace enrichment on immunosorbents for the multiresidue analysis of pH and triazine pesticides. Journal of Chromatography A. 725(1): 107-119.

Pitcher, F.G. (1973) TH 6040: Bluegill (*Lepomis macrochirus*). (U.S. Agricultural Research Service, Pesticides Regulation Div., Animal Biology Laboratory, unpublished study; CDL:132525-A). Cited in U.S. EPA 1997a.

Pouwelse A. 1986. Addendum to: Residues of Diflubenzuron in Water Soil, Sediment, Leaf Litter, Leaves and Residues of 4-Chlorophenylurea, A Breakdown Product of Diflubenzuron, in Soil From a Dissipation Study in a Forestry Area: Lab Project Number: 85-11: O.303.60.704. MRID 41922201.

Prendergast BF; Yendol WG; Maczuga S; Reardon RC; Mclane WH; Miller DR; Mcaneney MP. 1995. Diflubenzuron residue and persistence on an oak forest after aerial application. Journal of Environmental Science and Health Part B Pesticides Food Contaminants and Agricultural Wastes. 30(3): 359-376.

Quarles JM; Norman JO; Kubena LF. 1980. Absence of transformation by diflubenzuron in a host-mediated transplacental carcinogen assay. Bulletin of Environmental Contamination and Toxicology 25: 252-256.

Rabeni C; Gibbs K. 1975. The Effects of Dimilin on Non Target Stream Insects in Maine, 1975. Unpublished study prepared by University of Maine at Orono, Dept of Entomology. 7 p. MRID 00159905.

Rao PA; Mehrotra KN. 1997a. Effect of diflubenzuron on the AC resistance of the cuticle of *Schistocerca gregaria* Forskal. Journal of Entomological Research (New Delhi). 21(1): 39-44.

Rao PA; Mehrotra KN. 1997b. Influence of diflubenzuron on chitin and its consequential effect on electrical resistance of the cuticle of *Schistocerca gregaria* Forskal. Journal of Entomological Research (New Delhi). 21(3): 253-257.

Rao PA; Mehrotra KN; Jain MC. 1997. Effect of diflubenzuron on the capacitance of the cuticle of *Schistocerca gregaria* Forskal. Journal of Entomological Research (New Delhi). 21(2): 143-146.

Ravensberg, W.J. 1981. The natural enemies of the woolly apple aphid *Eriosoma lanigerum* Hausm. (Homoptera: Aphididae) and their susceptibility to diflubenzuron. Mededelingen van Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 46(2):437-441. Cited in USDA 1995.

Reardon RC. 1995a. Editor. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995. 174 pp.

Reardon RC. 1995b. Chapter 1. Introduction, pp. 2-5 in Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Reardon RC. 1995c. Chapter 18. Summary. pp. 168-174 in: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Rebach S. 1996. Effects of Dimilin on the blue crab *Callinectes sapidus* in shallow water habitats. J Shellfish Res. 15: 489.

Rebach S; French DP. 1996. Effects of Dimilin on the blue crab, *Callinectes sapidus*, in shallow-water habitats. Estuaries. 19(2A): 279-287.

Redfern RE; Demilo AB; Borkovec AB. 1980. Large milkweed bug: effects of diflubenzuron and its analogues on reproduction. Journal of Economic Entomology 73:682-683. Cited in USDA 1995.

Reiner HK; Parke GSE. 1975. Report: Static 96-Hour Toxicity Study of Thompson- Hayward Chemical Company. Sample TH 6040 in Fathead Minnows: Laboratory No. 5E-6095.

(Unpublished study received Jul 31, 1978 under 148-1259; prepared by Cannon Laboratories, Inc., submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL:234513-S). MRID 00060376. Cited in U.S. EPA 1997a.

Ribo JM; Kaiser KLE. 1984. Toxicities of chloranilines to *Photobacterium phosphoreum* and their correlations with effects on other organisms and structural parameters. In: Kaiser KLE, ed. QSAR environmental toxicology. Dordrecht, Reidel Publishing Company, pp. 319.336. Cited in WHO 2003.

Richmond ML; Henny CJ; Floyd RL; Mannan RW; Finch DM. 1979. Effects of sevin-4-oil, Dimilin, and orthene on forest birds in northeastern Oregon. NTIS/PB81-237281.

Ridgway RL; Thorpe KW; Webb RE; Venables L. 1994. Gypsy moth management in suburban parks: program evaluation. Journal of Entomological Science. 29(4): 557-569.

Riedl H; Hoying SA. 1980. Impact of fenvalerate and diflubenzuron on target and nontarget arthropod species on Bartlett pears in northern California. J. Econ. Entomol. 73(1): 117-122.

Roberts S; Parke GSE. 1976. Acute Oral Toxicity in Mallard Ducks: Laboratory No. 6E-2430 B. (Unpublished study received Dec 23, 1976 under 148-1258; prepared Cannon Laboratories, Inc., submitted by Thompson-Hayward Chemical Co., Kansas City, Kans.; CDL:095663-O). MRID 00073936.

Robinson F. 1978. The Effect of Repeated Spray Applications of Dimilin W-25 on Honey Bee Colonies in Cotton Fields. (Unpublished study received Dec 15, 1978 under 148-EX-25; prepared by Univ. of Florida, Institute of Food and Agricultural Sciences, Dept. of Entomology & Nematology, submitted by Thompson-Hayward Chemical Co., Kansas City, KS; CDL:097738-C). MRID 00099.

Robinson, F.A. 1979. The effects of repeated spray applications of Dimilin W-25 on honeybee (*Apis mellifera*) colonies in cotton fields (Toxicity, effects on brood rearing). American Bee Journal 119(3):193-194. Cited in USDA 1995.

Robinson, W.S.; Johansen, C.A. 1978. Effects of control chemicals for douglas-fir Tussock moth *Orgyia pseudotsugata* (McDonnough) on forest pollination (Lepidoptera: Lymantriidae). Melanderia 30:10-56. Cited in USDA 1995.

Rodriguez E; Barrio RJ; Goicolea A; Peche R; Gomez De Balugera Z; Sampedro C. 2001. Persistence of the insecticide Dimilin 45 ODC on conifer forest foliage in an Atlantic-climate ecosystem. Environ Sci Technol. 35(18):3804-8.

Romano M; Biroc S; Colavita J; et al. 1985. A Tissue Residue Study Utilizing a Vigilante 0.77% Diflubenzuron. (DFB) Pre- mix in Cattle when Fed at a Rate of 0.2 mg DFB/kg Body Weight. Unpublished study prepared by American Cyanamid Co. 46 p. MRID 00155419.

Ross DB; Prentice DE; Newman AJ; et al. 1977. Du 112307: 13 Weeks Oral Toxicity Study in the Sheep: PDR 229/77226. (Unpublished study received June 22, 1977 under 6F1773; prepared by Huntingdon Research Centre, England, submitted by Thompson- Hayward Chemical Co., Kansas City, Kans.; CDL:096167-H). MRID 00070034. Also summarized in WHO 1996.

Rozman K, Mueller W, Coulston F, et al. 1979. The involvement of the lymphatic system in the absorption, transport, and excretion of hexachlorobenzene in rats and rhesus monkeys. Toxicol Appl Pharmacol 48:A93.

Rumpf S; Frampton C; Chapman B. 1997a. Acute toxicity of insecticides to *Micromus tasmaniae* (Neuroptera: hemerobiidae) and *Chrysoperla carnea* (Neuroptera: chrysopidae): LC_{50} and LC_{90} estimates for various test durations. J Econ Entomol. 90: 1493-1499.

Rumpf S; Hetzel F; Frampton C. 1997b. Lacewings (Neuroptera: hemerobiidae and chrysopidae) and integrated pest management: enzyme activity as biomarker of sublethal insecticide exposure. J Econ Entomol. 90: 102-108.

Rumpf S; Frampton C; Dietrich DR. 1998. Effects of conventional insecticides and insect growth regulators on fecundity and other life-table parameters of *Micromus tasmaniae* (Neuroptera: hemerobiidae). J Econ Entomol. 91: 34-40.

Saleem MA; Shakoori AR; Falkous G; Wilkins RM; Mantle D. 1995. *In vitro* inhibition of proteolytic enzymes of human liver, kidney, brain and muscle tissues due to insecticides. Pakistan Journal of Zoology. 27(2): 95-103.

Sample BE; Cooper RJ; Whitmore RC. 1993. Dietary shifts among songbirds from a diflubenzuron-treated forest. Condor. 95 (3): 616-624.

Sample BE; Butler L; Whitmore RC. 1993. Effects of an operational application of Dimilin on non-target insects. Can Entomol. 125(2): 173-179.

Satake KN; Yasuno M. 1987. The effects of diflubenzuron on invertebrates and fishes in a river. Japanese Journal of Sanitary Zoology. 38 (4): 303-316.

Sauphanor B; Chabrol L; D'Arcier FF; Sureau F; Lenfant C. 1993. Side effects of diflubenzuron on a pear psylla predator: *Forficula auricularia*. Entomophaga. 38:163-174. Cited in USDA 1995.

Sauphanor B; Brosse V; Bouvier JC; Speich P; Micoud A; Martinet C. 2000. Monitoring resistance to diflubenzuron and deltamethrin in French codling moth populations (*Cydia pomonella*). Pest Manag Sci. 56: 74-82.

Savitz JD; Wright DA; Smucker RA. 1994. Toxic effects of the insecticide diflubenzuron (Dimilin) on survival and development of nauplii of the estuarine copepod, *Eurytemora affinis*. Marine Environmental Research. 37(3): 297-312.

Schaefer CH; Dupras Ef Jr. 1976. Factors affecting the stability of Dimilin in water and the persistence of Dimilin in field waters. J Agric Food Chem. 24(4): 733-739.

Schaefer CH; Dupras Ef Jr. 1977. Residues of diflubenzuron (1-(4-chlorophenyl) -3- (2,6-difluorobenzoyl) urea) in pasture soil, vegetation, and water following aerial applications. J Agric Food Chem. 25(5): 1026-1030.

Schaefer CH; Dupras EF; Stewart RJ; Davidson LW; Colwell AE. 1979. The accumulation and elimination of diflubenzuron by fish. Bull Environ Contam Toxicol. 21(1-2): 249-254.

Schocken M; Lengen M; Donovan K et al. 2001. Response to EPA's Proposal to Establish a Buffer Zone for Dimilin Aerial Forestry Applications: Lab Project Number: 2001-137: DIM 001: WEI 864.05. Unpublished study prepared by Uniroyal Chemical Co., Inc. 97 p. MRID 45517001.

Schmidt C. 1989. Schadwirkung von Phenolen, Anilinen und Aliphaten auf Algen. In: Chemikaliengesetz Heft 8, Prüfung und Bewertung von Stoffen auf ihre Umweltverträglichkeit. Berlin, Umweltbundesamt, pp. 98-137. Summarized in WHO 2003.

Schmidt C; Schnabl H. 1988. Stoffbezogene Struktur- Wirkungsbeziehungen bei Biotesten. Vom Wasser. 70: 21.32. Summarized in WHO 2003.

Schroeder W. 1978a. Letter sent to J. Taylor dated Feb 13, 1978: Dimilin sprays applied to citrus, effect on bees. (U.S. Agricultural Research Service, Southern Region, Horticultural Research Laboratory; unpublished study; CDL:096960-A). MRID 00099731.

Schroeder W. 1978b. Diflubenzuron 25W Applied for *Diaprepes abbreviatus* (L.), Population Suppression: Effect on the Nontarget Arthropod Complex in a Florida Citrus Grove. (Unpublished study received Dec 15, 1978 under 148-EX-25; submitted by Thompson- Hayward Chemical Co., Kansas City, KS; CDL:097738-B). MRID 00099742.

Schroeder WJ. 1996. Diflubenzuron residue:reduction of *Diaprepes abbreviatus* (Coleoptera:curculionidae) neonates. Fla Entomol. 79: 462-463.

Schroeder WJ; Sutton RA; Beavers JB. 1980. *Diaprepes abbreviatus*: fate of diflubenzuron and effect on nontarget pests and beneficial species after application to citrus for weevil control. J Econ Entomol. 73(5): 637-638.

SCI-GROW, 2001. Screening Ground Water Model, Version 2.2. November 1, 2001. Environmental Fate and Effects Division, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C. Available at: http://www.epa.gov/oppefed1/models/water/scigrow_description.htm

Seidel GE; Whitmore RC. 1995. Effects of Dimilin application on white-footed mouse populations in a central Appalachian forest. Environmental Toxicology and Chemistry. 14(5): 793-799.

Selvik A; Hansen PK; Ervik A; Samuelsen OB. 2002. The stability and persistence of diflubenzuron in marine sediments studied under laboratory conditions and the dispersion to the sediment under a fish farm following medication. Sci Total Environ. 285(1-3):237-45.

SERA (Syracuse Environmental Research Associates, Inc.). 2001. Preparation of Environmental Documentation and Risk Assessments, SERA MD 2001-01a, draft dated July 2001. Syracuse Environmental Research Associates, Inc., Fayetteville, NY. Available at www.sera-inc.com.

SERA (Syracuse Environmental Research Associates, Inc.). 2003a. Documentation for Worksheets Version 2.04 - Human Health and Ecological Risk Assessments, SERA WSD 01-2.04, report dated February 25, 2003. Available at: www.sera-inc.com.

SERA (Syracuse Environmental Research Associates, Inc.). 2003. Documentation for the Use of GLEAMS (Version 3) and Auxiliary Programs in Forest Service Risk Assessments (Version 2.04), SERA TD 2004-02.04a, dated February 8, 2004. Available at: www.sera-inc.com.

Sexstone A. 1995. Chapter 14. Microorganisms in Soil. pp. 130-139 in: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Sinha, S.N.; Lakhani, K.H.; Davis, N.K. 1990. Studies on the toxicity of insecticidal drift to the first instar larvae of the Large White butterfly *Pieris brassicae* (Lepidoptera: Pieridae). Annals of Applied Biology 116:27-41. Cited in USDA 1995.

Sindhu S; Muraleedharan D. 1997. Dimilin-induced endocrine changes in the ventral nerve cord ganglia of the red cotton bug *Dysdercus cingulatus* (Fabr) (Heteroptera : pyrrhocoridae). Proceedings of the Indian National Science Academy Part B Biological Sciences. 63(6): 551-558.

Smalley, H.E. 1976. Comparative toxicology of some insect growth regulators. Clinical Toxicology 2: 27.

Smith RP. 1996. Toxic Responses of the Blood. In: Casarett and Doull's Toxicology: The Basic Science of Poisons. 5th Edition. McGraw-Hill, Health Professions Division, New York, NY. pp. 335-353.

Soltani N; Soltani-Mazouni N. 1997. Oogenesis in mealworms: Cell density of germarium, thickness of chorion and ecdysteroid production: Effects of regulators. Med Fac Lanbouww, Univ Gent. 62(2b): 565-571.

Soltani N; Soltani-Mazouni N; Quennedey B; Delachambre J. 1996. Protein synthesis in developing ovaries of mealworm under in vivo and in vitro conditions: effects of diflubenzuron. J Stored Prod Res. 32: 205-212.

Soltani-Mazouni N; Soltani N. 1994a. Dosage quantitatif et qualitatif des proteines du corps gras, de l'hemolymphe et des ovaires au cours de la maturation sexuelle de *Tenebrio Molitor*: Effect du diflubenzuron et du Jeune. Med Fac Lanbouww, Univ Gent. 59(2a): 473-480.

Soltani-Mazouni N; Soltani N. 1994b. Deflubenzuron affected DNA synthesis in the ovaries of *Tenebrio Molitor*. Invertebrate Reproduction and Development. 25(1): 19-21.

Soltani-Mazouni N; Soltani N. 1995a. Protein synthesis in the fat body of *Tenebrio Molitor*(L.) During oocyte maturation: Effect of diflubenzuron, cycloheximide and starvation. J Stored Prod Res. 31(2): 117-122.

Soltani-Mazouni N; Soltani N. 1995b. Effet du diflubenzuron en traitement *in vivo* et *in vitro* sur la morphometrie de l'ovaire de *Tenebrio Molitor*(L.). Med Fac Lanbouww, Univ Gent. 60(3b): 961-967.

Spectrum Laboratories. 2004. *p*-chloroaniline Fact Sheet. Available at: http://www.speclab.com/.

Stephens L; McClane W; Wooldridge AW; et al. 1975. Effectiveness Data. (Unpublished study received Mar 8, 1976 under 239-EX-79; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:227742-E). (Unpublished study received Mar 8, 1976 under 239-EX-79; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:227742-E). MRID 00014931.

Stevenson JH. 1978. The acute toxicity of formulated pesticides to worker honey bees. (*Apis mellifera* L. Plant Pathol, 27: 38-40. Summarized in WHO 1997.

Stoner, A.; Wilson, W.T. 1982. Diflubenzuron (Dimilin): Effect of long-term feeding of low doses in sugar-cake or sucrose syrup on honey bees in standard-size field colonies. American Bee Journal 122(8):579-582. Cited in USDA 1995.

Story D. 1999. Comparison of Methemoglobin Formation and Splenic Tumor Incidence in Rats Fed para-chloroaniline or Diflubenzuron. Unpublished study prepared by Uniroyal Chemical Company. 5 p. MRID 44871304.

Stout MJ; Rice WC; Riggio RM; Ring DR. 2000. The effects of four insecticides on the population dynamics of the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel. J Entomol Sci. 35: 48-61.

Stribling HC; Smith HR. 1987. Effects of dimilin on diversity and abundance of forest birds. North J Appl For. 4: 37-38.

Sundaram KMS. 1986. Persistence and degradation of diflubenzuron in conifer foliage, forest litter and soil, following simulated aerial application. NTIS/MIC-93-02794.

Sundaram KMS. 1991. Spray deposit patterns and persistence of diflubenzuron in some terrestrial components of a forest ecosystem after application at three volume rates under field and laboratory conditions. Pestic Sci. 32: 275-293.

Sundaram KMS. 1996. Initial deposits, persistence and degradation kinetics of the insect growth regulator, diflubenzuron, in some terrestrial matrices following simulated aerial application. J Environ Sci Health, Part B, Pestic Food Contam Agric Wastes. (2): 135-163.

Sundaram KMS; Nott R. 1989. Mobility of diflubenzuron in two types of forest soils. J Environ Sci Health Part B Pestic Food Contam Agric Wastes. 24(1): 65-86.

Sundaram KMS; Sundaram A. 1994. Rain-washing of foliar deposits of Dimilin WP-25 formulated in four different carrier liquids. J Environ Sci Health, Part B, Pestic Food Contam Agric Wastes. (4): 757-783.

Sundaram KMS; Holmes SB; Kreutzweiser DP; Sundaram A; Kingsbury PD. 1991. Environmental persistence and impact of diflubenzuron in a forest aquatic environment following aerial application. Arch Environ Contam Toxicol. 20: 313-324.

Sundaram KMS; Sloane L; Nott R. 1997. Adsorption and desorption kinetics of diflubenzuron and fenitrothion in two different boreal forest soils. J Environ Sci Health, Part B, Pestic Food Contam Agric Wastes. (1): 1-24.

Sundaram K. 1986. Persistence and Degradation of Diflubenzuron in Conifer Foliage, Forest Litter and Soil, following Simulated Aerial Application. Information Report FPM-X. Unpublished study by Canadian Forestry Service. 35 p. MRID 00161955.

Sundari MSN; Arivalagan M; Balachander A; Durairaj G. 1998. Effect of diflubenzuron on the chitinase activity of predatory insect, *Cryptolaemus montrouzieri*. Journal of Environmental Biology. 19: 323-324.

Surprenant D. 1988. The Chronic Toxicity of Carbon[14]-Diflubenzuron to *Daphnia magna* under Flow-through Conditions: Laboratory Project ID: 11493.0188.6109.130. Unpublished study prepared by Springborn Life Sciences, Inc. 54 p. MRID 40840501.

Surprenant D. 1989. Acute Toxicity of Diflubenzuron to Quahogs (*Mercenaria mercenaria*) Embryo-Larvae Under Static Conditions. Lab Project Number: 89-3-2945: C 303.51.009. Unpublished study prepared by Springborn Laboratories, Inc. 25 p. MRID 41392001.

Swift MC; Smucker RA; Cummins KW. 1988. Effects of Dimilin on freshwater litter decomposition. Environ Toxicol Chem. 7 (2): 161-166.

Takahashi RM; Miura T. 1975. Multiple applications of Dimilin and Altosid to *Gambusia affinis* (Baird and Girard). Proceedings of the California Mosquito Control Association 43:85-87. MRID 00010645. [CBI03, GET1]

Tanner DK; Moffett MF. 1995. Effects of diflubenzuron on the reproductive success of the bluegill sunfish, *Lepomis macrochirus*. Environ Toxicol Chem. 14(8): 1345-55.

Technology Sciences Group Inc. 1997. Diflubenzuron: An Ecological Risk Assessment. Diflubenzuron: An Ecological Risk Assessment to Support an Expanded Label on Citrus: Lab Project Number: 96108. Unpublished study prepared by Uniroyal Chemical Co., Inc. 276 p. MRID 44460701.

Technology Sciences Group Inc. 1998. Diflubenzuron: An Ecological Risk Assessment: Supporting Published and Unpublished Reports: Lab Project Number: BW-87-3-2348: BW-87-5-2400: 11493-0886-6100-530. Unpublished study. 417 p. MRID 44460702.

Tembhare DB; Shinde JS. 1998. Effect of Dimilin on cephalic neuroendocrine organs in the larvae of fruit-sucking moth *Othreis materna* (Linn.) (Lepidoptera: noctuidae). Indian Journal of Experimental Biology. 36(3): 287-291.

Tester PA; Costlow J D JR. 1981. Effect of insect growth regulator Dimilin (TH 6040) on fecundity and egg viability of the marine copepod *Acartia tonsa*. Marine Ecology Progress Series. 5(3): 297-302.

Thompson S; Swigert J. 1993a. Diflubenzuron: A 5-Day Toxicity Test with the Freshwater Alga (*Anabaena flos-aquae*). Final Report: Lab Project Number: 225A-105C: 56835/21/93: 40208. Unpublished study prepared by Wildlife International Ltd. 93 p. MRID 42940102.

Thompson S; Swigert J. 1993b. Diflubenzuron: A 14-Day Toxicity Test with Duckweed (*Lemma gibba*): Final Report: Lab Project Number: 225A-103A: 56835/22/93: 40209. Unpublished study prepared by Wildlife International Ltd. 115 p. MRID 42940103.

Thompson S; Swigert J. 1993c. Diflubenzuron: A 5-Day Toxicity Test with the Freshwater Alga. (*Selenastrum capricornutum*): Final Report: Lab Project Number: 225A-102D: 56835/23/93: 40205. Unpublished study prepared by Wildlife International Ltd. 93 p. MRID 42940104.

Thompson S; Swigert J. 1993d. Diflubenzuron: A 5-Day Toxicity Test with the Freshwater Diatom (*Navicula pelliculosa*). Final Report: Lab Project Number: 225A-104A: 56835/24/93: 40207. Unpublished study prepared by Wildlife International Ltd. 93 p MRID 42940105.

Thompson S; Swigert J. 1993e. Diflubenzuron: A 5-Day Toxicity Test with the Marine Diatom (*Skeletonema costatum*). Final Report: Lab Project Number: 225A-101: 56835/25/93: 40206. Unpublished study prepared by Wildlife International Ltd. 109 p. MRID 42940106.

Thompson HM; Wilkins S. 2003 The effects of IGRs on honeybee populations. VIII International Symposium on Hazards of Pesticides to Bees. Bologna, September 4-6, 2002. Available At: http:// www.entom.agrsci.unibo.it /Convegno%20api/Riass%20 Hazards%20oral.htm .

Thorpe KW; Ridgway RL; Webb RE. 1997. Effectiveness of diflubenzuron and *Bacillus thuringiensis* against gypsy moth populations. Northern J Appl For. 14: 135-140.

Thus J; van der Laan-Straathof J. 1994. Fate of Diflubenzuron in Two Model Ditch Systems: Lab Project Number: C.303.62.024: 96138: 56835/55/93. Unpublished study prepared by Solvay Duphar B.V. 41 p. MRID 44399307.

Thus J; Van Dijk N; Rompa-Van der Veldt C; et al. 1991. Anaerobic Aquatic Metabolism of Diflubenzuron: Lab Project No. 56635/34/91: C.303.62.010. Unpublished study prepared by Duphar B. V. 36 p. MRID 41837601.

Tian X; Sabbagh GJ; Cuperus GW; Gregory M. 1996. Evaluating potential environmental impact of insecticide applications in a boll weevil eradication program. Water Resour Bull. 32: 1027-1037.

Timmerman B; Van Eck M; Ruijten H. 1992. Characterization of Residues of (Carbon[14]) Diflubenzuron in Lactating Goat, Volume I of III: Metabolite Patterns: Lab Project Number: 56629/83/90: C.303.63.002. Unpublished study prepared by Solvay Duphar B.V. Pharma Analysis Dept. 153 p. MRID 42494201.

Touart LW; Rao KR. 1987. Influence of diflubenzuron on survival, molting, and limb regeneration in the grass shrimp, *Palaemonetes pugio*. In: Pollution Physiology of Estuarine Organisms; Symposium in Georgetown, SC, October 21-24, 1985. No. 17, pp. 333-349. Cited in USDA 1995.

Townshend JL; Pree DJ; Broadbent AB. 1983. Population development and reproduction of fungus- and bacterium-feeding nematodes in the presence of insect growth regulators. Journal of Nematology. 15(1): 105-110. Cited in USDA 1995.

Turnipseed, S.G.; Heinrichs, E.A.; Da Silva, R.F.P.; Todd, J.W. 1974. Response of soybean insects to foliar applications of a chitin synthesis inhibitor TH 6040. J. of Econ. Entomology 67:760-762. Cited in USDA 1995.

Uniroyal Chemical Co. 2000. Dimilin: Reduced Risk and Organophosphate Alternative Rationale: Lab Project Number: 2000-134. Unpublished study. 92 p. MRID 45248401.

U.S. EPA. 1990. Integrated Risk Information System (IRIS). Diflubenzuron. File 0227. http://www.epa.gov/iris/subst/0227.htm. Last updated Sept. 1, 1990.

U.S. EPA. 1995. Integrated Risk Information System (IRIS). *p*-Choroaniline. File 0320. http://www.epa.gov/iris/subst/0320.htm. Last updated Feb 1, 1995.

U.S. EPA/OPP (U.S. Environmental Protection Agency/Office of Pesticide Programs). 1997a. Reregistration Eligibility Decision (RED): Diflubenzuron. EPA 738-R-97-008 dated August 1997. Available at: http://cfpub.epa.gov/oppref/rereg/status.cfm? show=rereg.

U.S. EPA/OPP. 1997b. R.E.D. Facts: EPA-738-F-97-008 dated August 1997. Available at: http://cfpub.epa.gov/oppref/rereg/status.cfm?show=rereg.

U.S. EPA/OPP. 1999. Diflubenzuron; Pesticide Tolerances. Federal Register. 64(74): 19050-19057. April 19, 1999. Available at: http://pmep.cce.cornell.edu/profiles/insect-mite/ddt-famphur/diflubenzuron/Diflubenzuron_tol_499.html.

US EPA/OPP. 2000a. Diflubenzuron; Pesticide Tolerances. Federal Register. 65(151): 47877-47882. August 4, 2000. Available at: http://www.epa.gov/fedrgstr/.

US EPA/OPP. 2000c. Administrative Record for the RED for Diflubenzuron (Case 0144). Unpublished compilation. 2028 p. MRID 45025301.

U.S. EPA/OPP. 2002a.   Diflubenzuron; Pesticide Tolerances for Emergency Exemption. Federal Register. 67(183): 59177-59182. September 20, 2002.   Available at: http://www.epa.gov/fedrgstr/EPA-PEST/2002/September/Day-20/p23819.htm.

U.S. EPA/OPP. 2002b. Cancellation of Pesticides for Non-payment of Year 2002 Registration Maintenance Fees. Federal Register. 67(183): 51250-51260. August 7, 2002.   Available at: http://pesticide.net/x/fedreg/2002/EPA-20020807B.html.

U.S. EPA/OPP. 2003.   Diflubenzuron; Pesticide Tolerances for Emergency Exemption. Federal Register. 68(166): 51479-51484. August 27, 2003.   Available at: http://www.epa.gov/fedrgstr/EPA-PEST/2003/August/Day-27/p21935.htm.

U.S. EPA/OPP (U.S. Environmental Protection Agency/Office of Pesticide Programs). 2004. Lists of Inert Pesticide Ingredients. Http://www.epa.gov/opprd001/inerts/. Last updated on Wednesday, January 7th, 2004

U.S. EPA/OPPTS (U.S. Environmental Protection Agency/Office of Prevention, Pesticides, and Toxic Substances). 2004. Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs, Endangered and Threatened Species Effects Determinations. Available at http://www.epa.gov/oppfead1/endanger/consultation/ ecorisk-overview.pdf.

U.S. EPA (U.S. Environmental Protection Agency). 1989a. Recommendations for and Documentation of Biological Values for use in Risk Assessment. U.S. EPA, Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH. ECAO-CIN-554. [pagination not continuous].

U.S. EPA/ORD (U.S. Environmental Protection Agency/Office of Research and Development). 1992. Dermal Exposure Assessment: Principles and Applications. EPA/600/8-91/011B. Interim Report. Exposure Assessment Group, Office of Health and Environmental Assessment, Washington, DC.

U.S. EPA/ORD (U.S. Environmental Protection Agency/Office of Research and Development). 1993. Wildlife Exposure Factors Handbook. Volumes 1 and 2. EPA/600/R-93/187a,b. Pagination not continuous. Available NTIS: PB94-174778 and PB94-174779.

USDA (U.S. Department of Agriculture). 1995. Gypsy Moth Management in the United States: A Cooperative Approach. Final Environmental Impact Statement. Appendix F (Human Health Risk Assessment) and Appendix G (Ecological Risk Assessment).

USDA/APHIS (U.S. Department of Agriculture Animal and Plant Health Inspection Service). 1993. Nontarget Risk Assessment for the MEDFLY Cooperative Eradication Program. USDA Animal and Plant Health Inspection Service. February 1993.

USDA/ARS (U.S. Department of Agriculture Agricultural Research Station). 1995. ARS Pesticide Properties Database. Http://wizard.arsusda.gov/rsml/testfiles. Listing last updated May 1995.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1989a. Final Environmental Impact Statement: Vegetation Management in the Coastal Plain/Piedmont, Management Bulletin R8-MB-23, dated January, 1989. 1213 pp.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1989b. Draft Environmental Impact Statement: Vegetation Management in the Ozark/Ouachita Mountains, Management Bulletin R8-MB-23, dated June, 1989. 499 pp.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1989c. Final Environmental Impact Statement: Vegetation Management in the Appalachian Mountains, Management Bulletin R8-MB-38, dated July, 1989. 1104 pp.

USDA/FS (U.S. Department of Agriculture/Forest Service). 2004. Diflubenzuron Usage by State. www.fs.fed.us/wv/gmdigest.

USGS (U.S. Geological Survey). 1998. Data on Pesticides in Surface and Ground Water of the United States., Results of the National Water Quality Assessment Program (NAWQA). Revised Oct. 23, 1998. http://wwwdwatcm.wr.usgs.gov/cppt/pns_data/data.html.

Van Berkel M; Van Eck M; De Bree H et al. 1986. Metabolite Patterns of [Carbon[14] Diflubenzuron in Liver, Kidney and Excreta of Pigs after Multiple Oral Dosing: Lab Project Number: C.303.650: 56630/86/86. Unpublished study prepared by Duphar B.V. Analytical Dept. 19 p. MRID 42494203.

Van De Veire M; Smagghe G; Degheele D. 1996. Laboratory test method to evaluate the effect of 31 pesticides on the predatory bug, *Orius laevigatus* (Het.: anthocoridae). Entomophaga. 41(2): 235-243.

Van Den Berg G. 1986. Dissipation of Diflubenzuron Residues after Application of Dimilin WP-25 in a Forestry Area in N. Carolina (USA) and Some Ecological Effects. Report No. 56637/47/1986. Unpublished study prepared by Duphar B.V. 161 p. MRID 00163853.

van Hemmen JJ. 1992. Agricultural pesticide exposure data bases for risk assessment. Rev. Environ. Contam. Toxicol. 126: 1-85.

van Kampen W; Thus J. 1996. The Determination of 4-Chloroaniline in Technical Grade Diflubenzuron: Validation and Typical Batch Analysis: Lab Project Number: C.303.10.012: 56834/23/95: 727253. Unpublished study prepared by Solvay Duphar B.V. 87 p. MRID 44486401.

Vanstone C. 1998a. Preliminary Analysis for Diflubenzuron in Dimilin 4L, UBI 1758-02: Lab Project Number: 95164: GRL-10883. Unpublished study prepared by Uniroyal Chemical Co., Inc. 10 p. {OPPTS 830.1700}. MRID 44574304.

Vanstone C. 1998b. Preliminary Analysis for Diflubenzuron in Dimilin 25W, UBI 1665: Final Report: Lab Project Number: 95198. Unpublished study prepared by Uniroyal Chemical Co., Inc. 10p. {830.1700}. MRID 44574903.

Vanstone C. 1998c. Preliminary Analysis for Diflubenzuron in Dimilin 2L, UBI 4064-02: Final Report: Lab Project Number: GRL-FR-11341: GRL-11341: 98054. Unpublished study prepared by Uniroyal Chemical Co. 25 p. {OPPTS 830.1700}. MRID 44636301.

Veech, J.A. 1978. The effect of diflubenzuron on the reproduction of free-living nematodes. Nematologica 24:312-320. Cited in USDA 1995.

Viswanathan R 1984. Regenwurmtest. In: Ballhorn L, Freitag D, eds. Überprüfung der Durchführbarkeit von Prüfungsvorschriften und der Aussagekraft der Stufe I und II des E. Chem. G. Neuherberg, Gesellschaft für Strahlen-und Umweltforschung München mbH, pp. 124–131. Summarized in WHO 2003.

Wadleigh, R.W.; Koehler, P.G.; Patterson, R.S. 1991. Age-specific reduction in German cockroach (Blattoidea: Blattellidae) populations exposed to diflubenzuron. Journal of Entomological Science 26(2):244-252. Cited in USDA 1995.

Walker R. 1999. The significance of excursions above the ADI: duration in relation to pivotal studies. Regul Toxicol Pharmacol. 30(2 Pt 2): S114-8.

Walker AN; Horst MN. 1992. Effects of diflubenzuron on chitin synthesis in the postmolt blue crab *Callinectes sapidus*: A morphologic study using an *in vitro* explant culture system. J Crustacean Biol. 12(3): 354-360.

Walstra P; Joustra K. 1990. Aerobic Soil Metabolism of Diflubenzuron in Sandy Loam: Lab Project Number: C.303.62.015. Unpublished study prepared by Duphar B.V. 38 p. MRID 41722801.

Wan MTK; Wilson DM. 1977. Impact of Insect Growth Regulators on Selected Non-target Organisms Co-existing with Mosquito Larvae: Report No. EPS 5-PR-77-1. (U.S. Environmental Protection Service, Pacific Region, Pollution Abatement Branch; unpublished study; CDL:234512-U). MRID 00095416.

Wang CJK. 1975. Effects of Dimilin upon microorganisms in leaf litter and forest soil. In: Evaluation of Dimilin against the Gypsy moth effects on non-target organisms 1975. Report expanded Gypsy moth research and applications program, Hamden USA (Unpublished report No. NTP-18, submitted to WHO by Solvay Duphar BV, Weesp, The Netherlands). Summarized in WHO 1996.

Wang S; Allan RD; Skerritt JH; Kennedy IR. 1998. Development of a class-specific competitive ELISA for the benzoylphenylurea insecticides. J Agric Food Chem. 46: 3330-3338.

Webb RE; Shapiro M; Podgwaite JD; Reardon RC; Tatman KM; Venables L; Kolodny-Hirsch DM. 1989. Effect of aerial spraying with Dimilin, Dipel, or Gypchek on two natural enemies of the gypsy moth (Lepidoptera: lymantriidae). J Econ Entomol. 82: 1695-1701.

Webb RE; Mclane WH; Finney JA; Venables L; White GB; Wieber AM; Cohen DL. 1994. Destruction of gypsy moth egg masses (Using surfactants, detergents, oils or conventional insecticides) for quarantine and community action programs. Journal of Entomological Science. 29(3): 305-317.

Webb RE; Peiffer R; Fuester RW; Thorpe KW; Calabrese L; Mclaughlin JM. 1998. An evaluation of the residual activity of traditional, safe, and biological insecticides against the gypsy moth. J Arboric. 24: 286-293.

Weiland R. 2000. Effects of Diflubenzuron and Other Products on Beneficials Resistance Investigations with Diflubenzuron and Other Products: A Compilation of Published Articles: Lab Project Number: DL-3574. Unpublished study prepared by Uniroyal Chemical Corporation. 1. MRID 45248403.

Weis JS; Ma A. 1987. Effects of the pesticide diflubenzuron on larval horseshoe crabs *Limulus polyphemus*. Bull Environ Contam Toxicol. 39 (2): 224-228.

Weis JS; Cohen R; Kwiatkowsi JK. 1987. Effects of diflubenzuron on limb regeneration and molting in the fiddler crab *Uca pugilator*. Aquat Toxicol (Amst). 10(5-6): 279-290.

Welp G; Brümmer GW. 1999. Effects of organic pollutants on soil microbial activity: the influence of sorption, solubility, and speciation. Ecotoxicology and Environmental Safety, 43:83.90. Cited in WHO 2003.

Westigard PH. 1979. Codling moth: control on pears with diflubenzuron and effects on nontarget pest and beneficial species. J. Econ. Entomol. 72(4): 552-554.

White WB. 1975. Evaluation of Dimilin against the Gypsy moth and effects on non-target organisms, 1975 (Compiled by the expanded Gypsy moth research and applications program). Upper Darby, Pennsylvania, US Department of Agriculture, Forest Service (Unpublished report). Summarized in WHO 1996.

White K. 1998. Product Identity and Composition of Diflubenzuron Technical: Final Report: Lab Project Number: 97069. Unpublished study prepared by Uniroyal Chemical Co., Inc. 4p. {830.1550}.   MRID 44574606.

Whitmore RC; Cooper RJ; Sample BE. 1993. Bird fat reductions in forests treated with Dimilin. Environ Toxicol Chem. 12: 2059-2064.

WHO (World Health Organization). 1996. Diflubenzuron. Environmental Health Criteria   184, 153 pp. Available at: http://www.inchem.org/documents/ehc/ehc/ehc184.htm.

WHO (World Health Organization). 2001. Diflubenzuron. Pesticide Residues in Food 2001. Toxicological Evaluations. Diflubenzuron. Available at: http://www.inchem.org/documents/jmpr/jmpmono/2001pr04.htm.

WHO (World Health Organization). 2003.   Concise International Chemical Assessment Document 48: 4-Chloroaniline. Available at: http://www.inchem.org/documents/cicads/cicads/cicad48.htm.

Wilcox H; Coffey T. 1978. Environmental Impacts of Diflubenzuron (Dimilin) Insecticide. By ERA Laboratories, Inc. S.l. : U.S. Forest Service, Forest Insect and Disease Management. MRID 00126458.

Willard T. 1999. Aquatic Field Soil Dissipation of Dimilin 2L (Diflubenzuron) in Rice in Arkansas and California. Final Report: Lab Project Number: 98012: AA980701: 734W. Unpublished study prepared by American Agricultural Services, Inc. and PTRL West, Inc. 754 p. MRID 45009601.

Willard T. 2000a. Dimilin 25W (Diflubenzuron): Non-Food Aquatic Field Dissipation and Bioaccumulation in Aquatic Non-target Organisms: Final Report: Lab Project Number: 98014: 727W: AA980702. Unpublished study prepared by American Agricultural Services, Inc. and PTRL Wes.   MRID 45191001.

Willard T. 2000b. Aquatic Field Soil Dissipation of Dimilin 2L (Diflubenzuron) in Rice in Arkansas and California: Lab Project Number: AA980701: 734W: 98012. Unpublished study prepared by American Agricultural Services, Inc.   MRID 45197601.

Willard T. 2000c. Dimilin 25W (Diflubenzuron): Non-Food Aquatic Field Dissipation and Bioaccumulation in Aquatic Non-target Organisms: Lab Project Number: AA980702: 727W: 98014. Unpublished study prepared by American Agricultural Services, Inc.   MRID 45197602.

Willcox H; Coffey T. 1978. Forest Insect and Disease Management: Environmental Impacts of Diflubenzuron (Dimilin) Insecticide. Prepared by ERA Laboratories, Inc., Broomall, PA: US Forest Service. 20 p. MRID 00159903.

Willems AGM; Overmars H; Scherpenisse P; Delange N; Post LC. 1980. Diflubenzuron: intestinal absorption and metabolism in the rat. Xenobiotica 10(2): 103-112.

Wilson JE. 1997. Age-specific sensitivity of grass shrimp (*Palaemonetes pugio*) embryos to sublethal concentrations of diflubenzuron. Environmental Toxicology and Risk Assessment: Modeling and Risk Assessment (ASTM STP 1317); 6:533-50.

Wilson J EH; Costlow JD. 1986. Comparative toxicity of two Dimilin formulations to the grass shrimp *Palaemonetes pugio*. Bull Environ Contam Toxicol. 36(6): 858-865.

Wilson J EH; Costlow JD. 1987. Acute toxicity of diflubenzuron to various life stages of the grass shrimp *Palaemonetes pugio*. Water Air Soil Pollut. 33(3-4): 411-418.

Wilson DM; Wan MTK. 1977. Effects of Orthene and Dimilin Insecticides on Selected Non-target Arthropods in a Douglas-fir Forest Environment: Report No. EPS-5-PR-76-4. (U.S. Environmental Protection Service, Pacific Region, Pollution Abatement Branch; unpublished study; CDL:234512-V). MRID 00095419 and MRID 00129973.

Wilson J EH; Forward R B JR; Costlow JD. 1999. Effects of diflubenzuron on the ontogeny of phototaxis by *Palaemonetes pugio*. Gulf Research Reports. 11(0): 7-14.

Wimmer MJ; Smith RR; Wellings DL; Toney SR; Faber DC; Miracle JE; Carnes JT; Rutherford AB. 1993. Persistence of diflubenzuron on Appalachian forest leaves after aerial application of Dimilin. Journal of Agricultural and Food Chemistry. 41(11): 2184-2190.

Wimmer MJ. 1995. Chapter 16. Terrestrial Environment. pp. 146-160 in: Reardon RC, Ed. Effects of Diflubenzuron on Nontarget Organisms in Broadleaf Forested Watersheds in the Northeast. USDA Nation Center of Forest Health Management. FHM-CN-05-95. November 1995.

Wirth S; Vogel K. 1988. Cow's milk protein intolerance in infants with methemoglobinemia and diarrhea. European Journal of Pediatrics (Berlin) 148(2):172. Cited in USDA 1995.

Wisniewska J; Prokopy RJ. 1997. Pesticide effect on faunal composition, abundance, and body length of spiders (*Araneae*) in apple orchards. Environmental Entomology. 26(4): 763-776.

Wittmann, D. 1982. Determination of the LC_{50} of Dimilin 25 WP for honey bee brood in free flying colonies as an example for the use of a new *Apis*-larvae test. Apidologie 13(1):104-107. Cited in USDA 1995.

Woebkenberg NR; Mostardi RA; Ely DL; Worstell D. 1981. Carboxyhemoglobin and methemoglobin levels in residents living in industrial and nonindustrial communities. Environmental Research 26(2):347-352. Cited in USDA 1995.

Wright DJ; Verkerk R HJ. 1995. Integration of chemical and biological control systems for arthropods: evaluation in a multitrophic context. Pesticide Science. 44(3): 207-218.

Wright DA; Savitz JD; Dawson R; Magee J; Smucker RA. 1996. Effect of diflubenzuron on the maturation and reproductive success of the copepod *Eurytemora affinis*. Ecotoxicology. 5(1): 47-58.

Yamano T; Morita S. 1993. Effects of pesticides on isolated rat hepatocytes, mitochondria, and microsomes. Archives of Environmental Contamination and Toxicology. 25(2): 271-278.

Yasuno M; Satake K. 1990. Effects of diflubenzuron and methoprene on the emergence of insects and their density in an outdoor experimental stream. Chemosphere. 21(10-11): 1321-1336.

Yu W. 1999. Determination of the Dissociation Constant of Diflubenzuron: Termination Final Report: Lab Project Number: GRL-FR-11521: GRL-11521: 99004. Unpublished study prepared by Uniroyal Chemical Co. 17 p. {OPPTS 830.7370}. MRID 44774205.

Yu SJ; Robinson FA; Nation JL. 1984. Detoxication capacity in the honey bee, *Apis mellifera* L. Pestic Biochem Physiol, 22: 360-368. Summarized in WHO 1996.

Zacarias MS; de Moraes JC; de Castro Diniz L; Ciociola AI; Damasceno AG. 1998. Selectivity of insect growth regulators to eggs and nymphs of Podisus nigrispinus (Dallas) (Homoptera: pentatomidae). Ciênc. E Agrotec., Lavras. 22(2): 194-198.

Zungoli PA; Steinhauer AL; Linduska JJ. 1983. Evaluation of diflubenzuron for Mexican bean beetle (Coleoptera: Coccinellidae) control and impact on *Pediobius foveolatus* (Hymenoptera: Eulophidae). Journal of Economic Entomology 76: 188-191. Cited in USDA 1995.

Table 2-1. Selected physical and chemical properties of diflubenzuron [1]

Synonyms and trade names	DFB; Difluron; Dimilin; Duphacid; DU 112307; ENT 29054; Micromite; OMS 1804; PH 60-40; TH-6040
U.S. EPA Reg. No.	400-465 and 400-474 (C&P Press, 2003)
CAS number	35367-38-5 (USDA/ARS 1995)
Molecular weight	310.69 (USDA/ARS 1995; Meylan and Howard 1995)
Molecular formula	$C_{14}H_9ClF_2N_2O_2$ (USDA/ARS 1995; Budavari 1989)
SMILES Notation	O=C(NC(=O)c(c(F)ccc1)c1F)Nc(ccc(c2)Cl)c2
Appearance/state, ambient	Solid (USDA/ARS 1995)
Melting point	230 to 232 °C (USDA/ARS 1995)
Vapor pressure	0.00012 mPa (USDA/ARS 1995)
Water solubility (mg/L)	≈0.3 (Budavari 1989) 0.08 at 25°C (USDA/ARS 1995; Knisel et al. 1992) 0.0888 mg/L in deionized, 0.0926 mg/L in field water (Mabury and Crosby 1996)
log K_{ow}	3.89 (USDA/ARS 1995) [i.e., $K_{ow} = 10^{3.89} = 7762$] 3.59 (estimated) (Meylan and Howard 1995) 3.88 (experimental) (Meylan and Howard 1995) 3.83 ±0.02 (Marsella et al. 2000)
Koc	135.3 (organic soil) (Sundaram et al. 1997) 332.0 (silty clay loam) (Sundaram et al. 1997) 8700 (NOS) (USDA/ARS 1995) 10000 (Knisel and Davis 2000)
Kd	17.59 (organic soil) (Sundaram et al. 1997) 16.42 (silty clay loam) (Sundaram et al. 1997)
Foliar halftimes	9.3 days (Sundaram 1986, 1996) 8 days, 20-80% loss (Wimmer et al. 1993[2]) 29 days (hardwood, van den Berg 1986) 36 days (conifer, van den Berg 1986)
Foliar washoff	50% to 100% depending on formulation, intensity of rainfall, and time of rain after application (Sundaram and Sundaram 1994)
Litter halftimes	8.36 days (Sundaram 1986, 1996)
Soil halftimes	sterile: 346 days in sand and muck (NOS)(Chapman et al. 1985) natural: 18.7 days in sand and muck (NOS)(Chapman et al. 1985) 7.49 days (field study, Sundaram 1986, 1996)
Water photolysis halftime	17±4 hours at pH 7 in distilled water (Marsella et al. 2000) 8±2 hours at pH 9 in distilled water (Marsella et al. 2000) 12.3±0.7 hours at pH 9 in stream water (Marsella et al. 2000)
Aerobic microbial halftime (soil/water)	25.7 days for DFB; 39.7 days for 4-chlorophenylurea (Dzialo and Maynard 1999) 50 hours [2.1 days] (Walstra and Joustra 1990) 5.4 days in water, 8.6 days in sediment (Willard 2000a)
Anaerobic microbial halftime (soil/water)	34 days (Thus et al. 1991)
Water halftime (NOS)	0.97 (0.77-1.16) days without aeration (Anton et al. 1993)
Henry's law constant	0.00047 Pa m³/mol at 25°C (USDA/ARS 1995) 0.234 ±0.002 Pa×m³/mole at 20°C (Mabury and Crosby 1996)

[1] Specific environmental fate parameters used in modeling are discussed in Section 3.2.
[2] Reflects initial losses. Remaining DFB much more persistent.

Table 2-2: Commercial formulations of diflubenzuron [1]

Formulation (Supplier)	Type of formulation	%DFB (w/w) [2] (Concentration)	Application Rates [3] Single	Application Rates [3] Total for year	Uses
Adept (Uniroyal)	Water Soluble Bags	25%	N/A	N/A	Ornamentals
Dimilin 2L (Uniroyal)	Aqueous flowable	22% (2 lbs/gallon)	2-16 fl oz/acre	24 fl oz/acre	Trees and various crops
Dimilin 4L (Uniroyal)	**Liquid**	**40.4 % (4 lbs/gallon)**	**0.5-2 fl oz/acre**	**2 fl oz/acre**	**Forests, ground or aerial.**
Dimilin 25W [4] (Uniroyal)	**Wettable powder**	**25%**	**1-4 oz/acre**	**4 oz/acre**	
Dimilin SC (Uniroyal)	Liquid	40.4 % (4 lbs/gallon)	N/A	N/A	Mushrooms and ornaments
Micromite 25W [5] (Uniroyal)	**Wettable powder**	**25%**	**1-4 oz/acre**	**4 oz/acre**	**Forests, ground or aerial.**
Micromite 25WS (Uniroyal)	Water Soluble Bags	25%	1.25 lbs/acre	3.75 lbs/acre	Citrus crops, ground or aerial
Micromite 25WGS (Uniroyal)	Water Dispersible Granules	80%	6.25 oz/acre	18.75 oz/acre	Citrus crops, ground or aerial

[1] Source: Specimen labels from C&P Press, 2004. Only products in bold font are labeled for gypsy moth.

[2] The remainder of the product formulation is classified as *inerts*. See text for discussion.

[3] All application rates are expressed in amount (lb or oz) of formulation not amounts of active ingredient per acre. N/A indicated that the product is not labeled for broadcast applications. For products labeled for gypsy moth, the range of application rates are those that apply to the gypsy moth.

[4] A separate formulation is available for mushrooms and ornamentals.

[5] The registration for this formulation has been canceled (U.S. EPA/OPP 2002b)

TABLE 2-3: Use of diflubenzuron by USDA from 1995 to 2002 for Suppression, Eradication, and Slow the Spread [1]

Year	Suppression	Eradication	Slow the Spread	Total
1995	161,231			161,231
1996	111,362	6	1,248	112,616
1997	16,447			16,447
1998	757			757
1999	5,275		1,047	6,322
2000	18,090			18,090
2001	187,784		650	188,434
2002	131,601		3,938	135,539
2003	25,124			25,124
Total Acres	657,671	6	6,883	664,560
% of Total	98.96%	0.001%	1.04%	

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

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

Chemical Specific Parameters

Parameter	Clay	Loam	Sand	Comment/Reference
Halftimes (days)				
Aquatic Sediment	34	34	34	Thus et al. 1991
Foliar	9.3	9.3	9.3	Sundaram 1986, 1996
Soil	10	1.1	2.1	Note 1
Water		5.4		Note 2
$K_{o/c}$, mL/g		8700		Note 3
K_d, mL/g	261	130	26.1	Note 4
Water Solubility, mg/L		0.0926		Mabury and Crosby 1996, field sample
Foliar wash-off fraction		0.5		Note 5
Fraction applied to foliage		0.8		
Fraction applied to soil		0.2		

Note 1   Value for sand taken as reported half-time of 50 hours (2.0833 days) taken from Walstra and Joustra 1990. Value for loam taken as reported half-time in silt-loam from Thus and van der Laan-Straathof 1994. No studies on aerobic soil metabolism in clay were found. The value of 10 days is taken from Knisel and Davis (2000) as an upper range.

Note 2   Value for microbial halftime in water from Willard 2000a. Halftimes may be substantially less under conditions where photolysis is the principal route of degradation. See Table 2-1.

Note 3   A very wide range of Koc values (about 135 to 10,000) have been reported (see Table 2-1). The value of 8700 is recommended by USDA/ARS (1995) and is close to the value of 10,000 recommended by Knisel and Davis (2000).

Note 4   Based on the general relationship: Kd = Koc × OC using OC values of 0.003 for sand, 0.015 for loam, and 0.030 for clay (SERA 2003b).

Note 5   This is highly variable. Knisel and Davis (2000) recommend 0.05. The higher value of 0.5 is consistent with the field studies by Sundaram and Sundaram (1994) and Wimmer et al. (1993).

Site Parameters
(see SERA 2004, TD 2004-02.04a dated February 8, 2004 for details)

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

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

Annual Rainfall (inches)	Rainfall per Event (inches)[1]	Clay		Loam		Sand	
		Average	Maximum	Average	Maximum	Average	Maximum
		Concentration per lb/acre applied (from GLEAMS)					
5	0.14	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
10	0.28	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
15	0.42	0.04113	5.17705	0.00000	0.00000	0.00000	0.00000
20	0.56	0.11543	14.59505	0.00000	0.00000	0.00000	0.00000
25	0.69	0.20602	26.22114	0.00000	0.00000	0.00000	0.00000
50	1.39	0.60485	81.46441	0.00000	0.00000	0.00000	0.00002
100	2.78	1.02559	156.23308	0.03588	11.68278	0.00000	0.00028
150	4.17	1.04171	199.48431	0.09107	29.67516	0.00000	0.00105
200	5.56	0.97117	229.82322	0.15544	50.70660	0.00001	0.00258
250	6.94	0.88544	253.52663	0.22002	71.88424	0.00045	0.13780
Application rate:		**0.0624**	lbs/acre				
		Concentration at above application rate					
5	0.14	0	0	0	0	0	0
10	0.28	0	0	0	0	0	0
15	0.42	0.003	0.32305	0	0	0	0
20	0.56	0.007	0.91073	0	0	0	0
25	0.69	0.0129	1.6362	0	0	0	0
50	1.39	0.0377	5.08338	0	0	0	0
100	2.78	0.064	9.74894	0.002	0.72901	0	0
150	4.17	0.065	12.4478	0.006	1.85173	0	0
200	5.56	0.0606	14.341	0.01	3.16409	0	0
250	6.94	0.0553	15.8201	0.0137	4.48558	0	0.009

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

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

Annual Rainfall (inches)	Rainfall per Event (inches)[1]	Clay Average	Clay Maximum	Loam Average	Loam Maximum	Sand Average	Sand Maximum
		Concentration per lb/acre applied (from GLEAMS)					
5	0.14	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
10	0.28	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
15	0.42	0.00704	0.07849	0.00000	0.00000	0.00000	0.00000
20	0.56	0.01700	0.26465	0.00000	0.00000	0.00000	0.00000
25	0.69	0.02989	0.56583	0.00000	0.00000	0.00000	0.00000
50	1.39	0.11171	3.32693	0.00000	0.00000	0.00000	0.00000
100	2.78	0.29257	12.37300	0.01577	1.63558	0.00000	0.00007
150	4.17	0.39616	23.59907	0.04933	5.81660	0.00000	0.00033
200	5.56	0.45379	35.86106	0.09695	12.41986	0.00001	0.00096
250	6.94	0.48619	48.35946	0.15210	20.70574	0.00035	0.05865
Application rate:	**0.0624**	lbs/acre					
		Concentration at above application rate					
5	0.14	0	0	0	0	0	0
10	0.28	0	0	0	0	0	0
15	0.42	0.0004	0.0049	0	0	0	0
20	0.56	0.00106	0.016514	0	0	0	0
25	0.69	0.00187	0.035308	0	0	0	0
50	1.39	0.00697	0.2076004	0	0	0	0
100	2.78	0.018256	0.7720752	0.001	0.1020602	0	0
150	4.17	0.02472	1.472582	0.00308	0.3629558	0	0
200	5.56	0.028317	2.2377301	0.00605	0.7749993	0	0
250	6.94	0.030338	3.0176303	0.00949	1.2920382	0	0.00366

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

Table 3-4: Chemical and site parameters used in GLEAMS modeling for 4-chloroaniline.

Chemical Specific Parameters				
Parameter	Clay	Loam	Sand	Comment/ Reference
Halftimes (days)				
Aquatic Sediment		150		Note 2
Foliar		0.16		Note 2
Soil		37.5		Note1
Water		151		Note 2
$K_{o/c}$, mL/g		72		Note 1
K_d, mL/g	2.2	1.1	0.22	Note 3
Water Solubility, mg/L		3900		Note 1
Foliar wash-off fraction		0.5		
Coefficient of transformation		0.41		Note 4

Note 1   Estimated from EPI-Suite (Meylan and Howard 1998, 2000)

Note 2   WHO 2003. Foliar halftime is not given explicitly in WHO (2003) and is estimated here based on the atmospheric halftime of 3.9 hours.

Note 3   Based on $Kd = K_{o/c} \times OC$, where OC is the proportion of organic carbon. The OC in sand, loam, and clay is taken as 0.003 for sand, 0.015 for loam, and 0.030 for clay (SERA 2004).

Note 4   This is the ratio of the molecular weight of chloroaniline (127.57) to that of diflubenzuron (310.69). See discussion by Knisel and Davis (2000, p. 110).

Table 3-5: Summary of modeled concentrations of 4-chloroaniline in streams (all units are µg/L or ppb)

Annual Rainfall (inches)	Rainfall per Event (inches)[1]	Clay		Loam		Sand	
		Average	Maximum	Average	Maximum	Average	Maximum
		Concentration per lb/acre applied (from GLEAMS)					
5	0.14	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
10	0.28	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
15	0.42	0.06559	4.19361	0.00048	0.01145	0.11234	2.57651
20	0.56	0.15452	10.45786	0.01616	0.32734	0.36403	10.48046
25	0.69	0.22436	15.84683	0.03969	0.85101	0.55917	19.16073
50	1.39	0.31156	27.90970	0.16080	4.59647	0.77622	44.23856
100	2.78	0.29226	30.80407	0.22906	9.17859	0.59128	52.72812
150	4.17	0.13293	24.52481	0.20128	9.67567	0.45074	51.02312
200	5.56	0.06009	14.09093	0.16267	8.73307	0.36145	49.79360
250	6.94	0.01924	5.74944	0.12680	7.21420	0.30139	47.06395
Application rate:		**0.0624**	lbs/acre				
		Concentration at above application rate					
5	0.14	0	0	0	0	0	0
10	0.28	0	0	0	0	0	0
15	0.42	0.00409	0.2616813	0	0.0007	0.00701	0.1607742
20	0.56	0.00964	0.6525705	0.00101	0.020426	0.022715	0.6539807
25	0.69	0.014	0.9888422	0.00248	0.053103	0.034892	1.1956296
50	1.39	0.019441	1.7415653	0.010034	0.2868197	0.048436	2.7604861
100	2.78	0.018237	1.922174	0.014293	0.572744	0.036896	3.2902347
150	4.17	0.00829	1.5303481	0.01256	0.6037618	0.028126	3.1838427
200	5.56	0.00375	0.879274	0.010151	0.5449436	0.022554	3.1071206
250	6.94	0.0012	0.3587651	0.00791	0.4501661	0.018807	2.9367905

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

Table 3-6: Summary of modeled concentrations of 4-chloroaniline in ponds (all units are µg/L or ppb)

Annual Rainfall (inches)	Rainfall per Event (inches)[1]	Clay Average	Clay Maximum	Loam Average	Loam Maximum	Sand Average	Sand Maximum
		Concentration per lb/acre applied (from GLEAMS)					
5	0.14	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
10	0.28	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
15	0.42	0.31929	0.69851	0.00288	0.00477	0.65741	1.11311
20	0.56	0.56688	1.80242	0.07465	0.15746	1.72523	4.20894
25	0.69	0.74573	2.90175	0.16734	0.40004	2.48876	7.61750
50	1.39	1.04158	6.43073	0.63473	2.28508	3.46266	18.05225
100	2.78	1.01591	8.41740	0.97319	5.00787	2.89735	23.03849
150	4.17	0.60259	6.77759	0.90309	5.52346	2.34727	22.92303
200	5.56	0.29679	4.08394	0.75792	5.16526	1.96069	22.29465
250	6.94	0.10055	1.77278	0.60774	4.47424	1.68309	21.01092
Application rate:		**0.0624**	lbs/acre				
		Concentration at above application rate					
5	0.14	0	0	0	0	0	0
10	0.28	0	0	0	0	0	0
15	0.42	0.019924	0.043587	0.0002	0.0003	0.041022	0.069458
20	0.56	0.035373	0.112471	0.00466	0.00983	0.1076544	0.2626379
25	0.69	0.046534	0.1810692	0.010442	0.024963	0.1552986	0.475332
50	1.39	0.064995	0.4012776	0.039607	0.142589	0.21607	1.1264604
100	2.78	0.063393	0.5252458	0.060727	0.3124911	0.1807946	1.4376018
150	4.17	0.037602	0.4229216	0.056353	0.3446639	0.1464696	1.4303971
200	5.56	0.01852	0.2548379	0.047294	0.3223122	0.1223471	1.3911862
250	6.94	0.00627	0.1106215	0.037923	0.2791926	0.1050248	1.3110814

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

Table 3-7: Estimated Environmental Concentrations (μg/L or ppb) of diflubenzuron in ponds and streams.

Scenario	Peak	Long-Term Average
MODELING FOR THIS RISK ASSESSMENT (0.0624 lb/acre or 70 g/ha)		
Stream		
Direct Spray [1]	5.7	N/A
100 Foot buffer [1]	0.11	N/A
GLEAMS (Table 3-2)	2 (<0.01 to 16)	0.01 (0 to 0.06)
Pond		
Direct Spray [2]	3.5	N/A
100 Foot buffer [2]	0.07	N/A
GLEAMS (Table 3-3)	0.2 (<0.005 to 3) at 0.06 lb/ac	0.007 (0 to 0.03) at 0.06 lb/ac
OTHER MODELING		
USDA (1995)	16.01 (stream, direct spray) 2.76 to 13.14 (stream, runoff) 1.22 (pond)	N/A
U.S. EPA/OPP 1997a. Pond: citrus crops	3.4 ppb at 6x 0.06 lb/ac 8.1 ppb at 0.67 lb/ac	0.74 ppb at 6x 0.06 lb/ac 0.87 ppb at 0.67 lb/ac
U.S. EPA/OPP 1997a. Pond: direct applications to water in forestry	11.7 ppb at 0.05 lb/ac 22.8 ppb at 0.07 lb/ac 46.2 ppb at 0.15 lb/ac 91.8 ppb at 0.32 lb/ac	N/A
Harned and Relyea 1997	Peak concentration of 1 ppb at an application rate of 350 g/ha. Longer term concentration of about 0.1 ppb. See text for discussion.	
Schocken et al. 2001	Peak concentrations of about 0.2 to 0.3 ppb in ponds and 0.9 ppb in steams at an application rate of 0.125 lb/acre. See text for discussion.	

[1] See Worksheet 10b
[2] See Worksheet 10a

Table 3-8: Concentrations of diflubenzuron in surface water used in this risk assessment (see Section 3.2.3.4.6 for discussion).

At application rate:	0.0624	lb/acre	
		Peak Concentration (ppb or µg/L)	Longer Term Concentration (ppb or µg/L)
	Central	0.4	0.02
	Lower	0.01	0.001
	Upper	16	0.1

Water contamination rate [1]		mg/L per lb/acre applied.	
		Peak Concentration (mg/L per lb/acre)	Longer Term Concentration (mg/L per lb/acre)
	Central	6.41e-03	3.21e-04
	Lower	1.60e-04	1.60e-05
	Upper	2.56e-01	1.60e-03

[1] Water contamination rates – concentrations in units of mg/L expected at an application rate of 1 lb/acre. These values are entered into Worksheet A04 for diflubenzuron. This rate is adjusted to the program application rate in all worksheets involving exposure to contaminated water.

Table 3-9: Concentrations of 4-chloroaniline in surface water used in this risk assessment (see Section 3.2.3.4.7 for discussion).

At application rate: **0.0624**   **lb/acre**

	Peak Concentration (ppb or µg/L)	Longer Term Concentration (ppb or µg/L)
Central	0.5	0.05
Lower	0.00003	0.0002
Upper	3	0.2

Water contamination rate [1]   **mg/L per lb/acre applied.**

	Peak Concentration (mg/L per lb/acre)	Longer Term Concentration (mg/L per lb/acre)
Central	8.01e-03	8.01e-04
Lower	4.81e-07	3.21e-06
Upper	4.81e-02	3.21e-03

[1] Water contamination rates – concentrations in units of mg/L expected at an application rate of 1 lb/acre. These values are entered into Worksheet A04 for 4-chloroaniline. This rate is adjusted to the program application rate in all worksheets involving exposure to contaminated water.

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

Range of Application Rates (g/ha)	Species	
	No Adverse Effects	Adverse Effects
<20	ants (Catangui et al. 1996) *Cotesia melanoscelus* (GM parasitic wasp) 　(Webb et al. 1989)	grasshoppers (Jech et al. 1993)
20 - <40	lacewing and beetles (Ables et al. 1977) carabids, crickets, lice (Butler et al. 1997) honey bee (Matthenius1975) honey bee [×8](Robinson 1978,1979)	gypsy moth and macrolepidoptera (Butler et al. 1997) grasshopper (Everts 1990) *Apanteles melanoscelus* # (GM parasitic wasp) 　(Madrid and Stewart1981)
40 - < 60	lacewing and beetles (Ables et al. 1977)	
60 - < 100	*Ooencyrtus kuvanae* (GM parasitic wasp) 　(Brown and Respicio 1981) lacewing and beetles (Deakle and Bradley1982) honey bee (Matthenius1975) sucking herbivorous insects, microlepidoptera, and 　predaceous arthropods(Martinat et al. 1988) spiders* and orthopteroid*(Martinat et al. 1993) mites and springtails (Perry et al. 1997) spiders** (Perry et al. 1997) non-lepidopteran insects (Sample et al. 1993a,b) mites* and collembolans* (Van Den Berg 1986)	grasshopper (Everts 1990) grasshoppers, moths, carabid beetles (Butler 1993) lepidoptera (Sample et al. 1993a,b) macrolepidoptera and other herbivorous insects 　(Martinat et al. 1988) Yellow jacket wasp (Barrows et al. 1994)
100 - < 150	ants (Weiland 2000) *Psylla* parasites and predators (Westigard 1979) lacewing and beetles (Ables et al. 1977) honey bee (Emmett and Archer 1980) honey bee [×8](Robinson 1978,1979)	soil mites (Blumberg 1986) Yellow jacket wasp (Weiland 2000)
150 - < 200	various arthropod predators (Keever et al. 1977)	lepidopteran egg mortality (low) (Kumar et al. 1994) mites (Marshall 1979)
200 - < 300	ants (Weiland 2000) carabid beetles (Heinrichs et al. 1979)	lacewing and beetles (Ables et al. 1977) mites (Marshall 1979) borer weevil (Schroeder 1996) predatory damsel bugs and sucking insects 　(Turnipseed et al. 1974) Yellow jacket wasp (Weiland 2000) *Psylla* parasites and predators (Westigard 1979) flying insects, esp. midges, gnats, and mosquitoes 　(Wilson and Wan 1977a)
≥ 300	honey bee (Buckner et al. 1975) honey bee (Emmett and Archer 1980) honey bee and other beneficial insects 　(Schroeder 1980)	lepidopteran egg mortality (high) (Kumar et al. 1994) *Psylla* parasites and predators (Westigard 1979)

[1] Studies summarized in Appendix 3a. See text for discussion. A single asterisk (*) indicates transient or equivocal effects. A double asterisk (**) indicates effects that were secondary to decrease in prey. The # symbol indicates an effect clearly due to toxicity. GM used as abbreviation for gypsy moth. Multiple applications are indicated in brackets with a × symbol followed by the number of applications.

Table 4-2: Summary of modeled concentrations of diflubenzuron in soil (all units are mg/kg or ppm)

Annual Rainfall (inches)	Rainfall per Event (inches)[1]	Clay		Loam		Sand	
		Average	Maximum	Average	Maximum	Average	Maximum
		Concentration per lb/acre applied (from GLEAMS)					
5	0.14	0.00841	0.14004	0.00092	0.11651	0.00169	0.12485
10	0.28	0.00926	0.14004	0.00106	0.11652	0.00194	0.12484
15	0.42	0.00924	0.13992	0.00106	0.11653	0.00193	0.12484
20	0.56	0.00918	0.13962	0.00106	0.11653	0.00193	0.12484
25	0.69	0.00910	0.13914	0.00106	0.11653	0.00193	0.12484
50	1.39	0.00834	0.13431	0.00106	0.11653	0.00192	0.12484
100	2.78	0.00650	0.11909	0.00104	0.11450	0.00190	0.12484
150	4.17	0.00412	0.09305	0.00099	0.10879	0.00188	0.12484
200	5.56	0.00234	0.06298	0.00091	0.09889	0.00186	0.12484
250	6.94	0.00104	0.05236	0.00080	0.08527	0.00184	0.12478
Application rate:		**0.0624**	lbs/acre				
		Concentration at above application rate					
5	0.14	5.2e-04	0.00874	5.7e-05	0.00727	1.1e-04	0.00779
10	0.28	5.8e-04	0.00874	6.6e-05	0.00727	1.2e-04	0.00779
15	0.42	5.8e-04	0.00873	6.6e-05	0.00727	1.2e-04	0.00779
20	0.56	5.7e-04	0.00871	6.6e-05	0.00727	1.2e-04	0.00779
25	0.69	5.7e-04	0.00868	6.6e-05	0.00727	1.2e-04	0.00779
50	1.39	5.2e-04	0.00838	6.6e-05	0.00727	1.2e-04	0.00779
100	2.78	4.1e-04	0.00743	6.5e-05	0.00714	1.2e-04	0.00779
150	4.17	2.6e-04	0.00581	6.2e-05	0.00679	1.2e-04	0.00779
200	5.56	1.5e-04	0.00393	5.7e-05	0.00617	1.2e-04	0.00779
250	6.94	6.5e-05	0.00327	5.0e-05	0.00532	1.1e-04	0.00779

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

Table 4-3: Summary of modeled concentrations of 4-chloroaniline in soil (all units are mg/kg or ppm)

Annual Rainfall (inches)	Rainfall per Event (inches)[1]	Clay		Loam		Sand	
		Average	Maximum	Average	Maximum	Average	Maximum
		Concentration per lb/acre applied (from GLEAMS)					
5	0.14	0.00672	0.02893	0.00680	0.04917	0.00750	0.04216
10	0.28	0.00655	0.02685	0.00626	0.04550	0.00666	0.04159
15	0.42	0.00699	0.02697	0.00709	0.04556	0.00751	0.04167
20	0.56	0.00691	0.02665	0.00734	0.04562	0.00728	0.04168
25	0.69	0.00668	0.02618	0.00748	0.04566	0.00685	0.04157
50	1.39	0.00360	0.02252	0.00737	0.04582	0.00493	0.04032
100	2.78	0.00631	0.01739	0.00622	0.04519	0.00323	0.04015
150	4.17	0.00307	0.01146	0.00529	0.04326	0.00254	0.04001
200	5.56	0.00142	0.00759	0.00450	0.03994	0.00216	0.03997
250	6.94	0.00050	0.00357	0.00375	0.03540	0.00193	0.03999
Application rate:		**0.0624**	lbs/acre				
		Concentration at above application rate					
5	0.14	4.2e-04	0.00181	4.2e-04	0.00307	4.7e-04	0.00263
10	0.28	4.1e-04	0.00168	3.9e-04	0.00284	4.2e-04	0.0026
15	0.42	4.4e-04	0.00168	4.4e-04	0.00284	4.7e-04	0.0026
20	0.56	4.3e-04	0.00166	4.6e-04	0.00285	4.5e-04	0.0026
25	0.69	4.2e-04	0.00163	4.7e-04	0.00285	4.3e-04	0.00259
50	1.39	2.2e-04	0.00141	4.6e-04	0.00286	3.1e-04	0.00252
100	2.78	3.9e-04	0.00109	3.9e-04	0.00282	2.0e-04	0.00251
150	4.17	1.9e-04	0.0007	3.3e-04	0.0027	1.6e-04	0.0025
200	5.56	8.9e-05	0.0005	2.8e-04	0.00249	1.3e-04	0.00249
250	6.94	3.1e-05	0.0002	2.3e-04	0.00221	1.2e-04	0.0025

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

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

Organism	Endpoint	Toxicity Value	Reference, Species
Mammals	Acute NOAEL	1118 mg/kg	Blaszcak 1997a, rats [Dimilin 2L]
	Chronic NOAEL	2 mg/kg/day	Greenough et al. 1985, dogs
Birds	Acute NOAEL	2500 mg/kg	Alsager and Cook 1975, blackbirds
	Chronic NOAEL	110 mg/kg	Beavers et al. 1990b, quail
Terrestrial arthropods		*See Table 4-5 for toxicity values*	
Soil invertebrates			
Earthworm	NOEC	780 mg/kg soil	Berends et al. 1992
Soil microorganisms			
Sensitive	50 ppm LOEC	50 ppm ÷ 10	Townshend et al. 1983
Tolerant	100 pp NOEC	100 ppm	Townshend et al. 1983
Fish Acute			
Sensitive	LC_{50}	25 mg/L	Johnson and Finley 1980, yellow perch
Tolerant	LC_{50}	500 mg/L	Reiner and Parke 1975, fathead minnow
Fish Chronic			
Sensitive	Reproductive NOEC	0.05 mg/L	Livingston and Koenig 1977, mummichog
Tolerant	Reproductive NOEC	0.1 mg/L	Cannon and Krize 1976, fathead minnow
Aquatic Invertebrates		*See Table 4-6 for toxicity values*	
Aquatic Plants			
Sensitive	NOEC for growth	0.045 mg/L	Hansen and Garton 1982a, *Selenastrum capricornutum*
Tolerant	NOEC for growth	0.38 mg/L	Thompson and Swigert 1993c, *Navicula pelliculosa*
Aquatic Microorganisms	NOEC for respiration	0.05 mg/L	Kreutzweiser et al. 2001 [4.3.3.4]

[1] NOECs are used directly when available. When only a LOEC is available, the LOEC is divided by 10 to approximate the NOEC. This is indicated by the "÷10" following the LOEC.

Table 4-5: Diflubenzuron toxicity values used in risk assessment for terrestrial arthropods (see Table 4-1 for additional details).

Organism	Endpoint	Toxicity Value[1]	Reference
Grasshoppers	Field LOAEL	22 g/ha ÷ 10	Jech et al. 1993
Apanteles melanoscelus[2]	Field LOAEL	30 g/ha ÷ 10	Madrid and Stewart1981
Macrolepidoptera	Field LOAEL	35 g/ha ÷ 10	Butler et al. 1997
Mandibulate herb. insects	Field LOAEL	70 g/ha ÷ 10	Martinat et al. 1988
Ooencyrtus kuvanae[2]	Field NOAEL	67 g/ha	Brown and Respicio 1981
Microlepidoptera	Field NOAEL	70 g/ha	Martinat et al. 1988
Predaceous arthropods	Field NOAEL	70 g/ha	Martinat et al. 1988
Sucking herbaceous insects	Field NOAEL/LOAEL	70/281 g/ha	Martinat et al. 1988/Turnipseed et al. 1974
Spiders	Field NOAEL	70 g/ha	Martinat et al. 1993
Mites and collembolans	Field NOAEL/LOAEL	70/140 g/ha	Perry et al. 1997/Blumberg 1986
ants	Field NOAEL	280	Weiland 2000
Lacewing	Field NOAEL/LOAEL	140/280 g/ha	Ables et al. 1977
Honey bee	Field NOAEL	400 g/ha	Emmett and Archer 1980

[1] Field NOAELs are used directly when available. When only a LOAEL is available, the LOAEL is divided by 10 to approximate the NOAEL. This is indicated by the "÷10" following the LOAEL.

[2] A parasitic wasp to the gypsy moth.

Table 4-6: Diflubenzuron toxicity values used in risk assessment for aquatic invertebrates.

Organism	Endpoint	Toxicity Value ppb or μg/L[1]	Reference
ACUTE (see Table 4-8 for additional details)			
Daphnia	NOEC	0.3	Corry et al. 1995
Ceriodaphnia	NOEC	0.75	Hall 1986
Copepods	NOEC	0.93	Savitz et al. 1994
crabs	NOEC	2	Cunningham and Meyers 1987
rotifers	NOEC	20	Corry et al. 1995
large insects	NOEC	2000	Lahr et al. 2001
molluscs	NOEC	125000	Wilcox and Coffey 1978
LONGER TERM (see Table 4-9 for additional details)			
Daphnia	NOEC	0.04	Surprenant 1988
stoneflies and mayflies	NOEC	0.1	Hansen and Garton 1982b
Ceriodaphnia	NOEC	0.25	Hall 1986
dragonflies	NOEC	0.7	O'Halloran and Liber 1995
ostracods	NOEC	2.5	Liber and O'Halloran 1995
coleoptera and oligochaetes	NOEC	50	Hansen and Garton 1982a
molluscs	NOEC	320	Surprenant 1989

[1] In worksheets, all concentrations in ppb are divided by 1000 to convert to concentrations in ppm or mg/L.

Table 4-7: Summary of 4-chloroaniline toxicity values used in ecological risk assessment

Organism	Duration/Endpoint	Toxicity Value	Reference, species
Mammals	Acute/Toxicity NOAEL	8 mg/kg/day	Used in HHRA
	Chronic/Toxicity NOAEL	1.25 mg/kg/day	Estimated from LOAEL of 12.5 mg/kg/day
Birds	Acute/Toxicity NOAEL	8 mg/kg/day	No data. Uses value for mammals
	Chronic/Toxicity NOAEL	1.25 mg/kg/day	No data. Uses value for mammals
Earthworms	NOEC	540 mg/kg soil	WHO 2003
Soil Microorganisms	NOEC	1000 ppm	Welp and Brummer 1999
Fish			
Acute	LC_{50}	2.4 mg/L	WHO 2003, Bluegill
Chronic	NOEC, reproduction	0.2 mg/L	Bresch et al. 1990, Zebra fish
Aquatic Invertebrates			
Acute	NOEC, mortality	0.013 mg/L	Kuhn et al 1989a
Chronic	NOEC, reproduction	0.01 mg/L	Kuhn et al 1989a
Aquatic plants	EC_{10}	0.02 mg/L	Schmidt and Schnabl 1988, green algae
Aquatic Microorganisms	NOEC (30 min)	5.1 mg/L	Ribo and Kaiser 1984, photobacteria

Table 4-8: Acute toxicity of diflubenzuron in aquatic invertebrates

Concentrations (µg/L or ppb)	No Effect Species/group [conc. ppb](Reference)	Adverse Effect Species/group [conc. ppb](Reference)
0.1 to <1	mysid shrimp[0.12] (Breteler 1987) *Daphnia* [0.3](Corry et al. 1995) *Daphnia* [0.45](Kuijpers 1988) *Ceriodaphnia* [0.75](Hall 1986) copepods [0.93](Savitz et al. 1994)	Mosquito [0.5] (Miura and Takahashi 1974) *Daphnia* [0.7](Corry et al. 1995) *Daphnia* [0.7](Kuijpers 1988) *Daphnia* [0.75, neonate](Majori et al. 1984) fairy shrimp [0.74] (Lahr et al. 2001)
1 to <10	fiddler crabs [2] (Cunningham and Meyers 1987) Horseshoe crabs[4] [5] (Weis and Ma 1987) amphipods [7] (Corry et al. 1995)	gammarids[1](Hansen and Garton 1982a) *Ceriodaphnia* [1.7](Hall 1986) copepods [1.7](Savitz et al. 1994) midges[1.8](Hansen and Garton 1982a) blue crab eggs [1.8] (Lee and Oshima 1998) grass shrimp [3.4](Tourat and Rao 1987) grass shrimp [2-3](Wilson and Costlow 1986) mysid shrimp[2.1]Nimmo et al. 1979
10 to <100	rotifers[20] (Corry et al. 1995) snails [45](Hansen and Garton 1982a)	Mayfly [10] (Miura and Takahashi 1974) Amphipods [13](Corry et al. 1995) *Daphnia* [23, adult](Majori et al. 1984) Dragonfly [50] (Miura and Takahashi 1974) Horseshoe crabs [50] (Weis and Ma 1987)
100 to <1000		beetles [100] (Miura and Takahashi 1974) fiddler crabs [200] (Cunningham and Meyers 1987) tricoptera [250] (Bradt and Williams 1990) grass shrimp[640] (Bionomics-EG&G 1975)
>1000	backswimmer[2] [2000] (Lahr et al. 2001) snail [125,000](Wilcox and Coffey 1978)	midge [560] (Julin and Sanders 1978)

[1] Macrocosm study

[2] No molting during short term exposures

[3] Litoral enclosures

[4] Marginal signs of toxicity

Table 4-9: Chronic toxicity of diflubenzuron in aquatic invertebrates

Concentrations (µg/L or ppb)	No Effect Species/group [conc. ppb](Reference)	Adverse Effect Species/group [conc. ppb](Reference)
>0.01 to 0.1	*Daphnia*[0.04]Surprenant 1988 stream inverts[1] [0.1](Hansen and Garton 1982a[1]) stoneflies and mayflies[0.1] (Hansen and Garton 1982b[1])	*Daphnia* [0.06] U.S. EPA 1997a [5] mysid shrimp[0.075]Nimmo et al. 1979 *Daphnia* [0.09]LeBlanc (1975) *Daphnia*[0.093]Surprenant 1988

>0.1 to 1	*Ceriodaphnia* [0.25](Hall 1986)
	mayflies, damselflies, and dragonflies[0.7]
	    (O'Halloran and Liber 1995)
	mixed insects[3] [1](Liber 1995)
		Ceriodaphnia [0.5](Hall 1986)
		clodacera and copopods[3][0.7]
		    (Liber and O'Halloran 1995)
		copepods [0.7-0.9](Wright et al. 1996)
		grass shrimp (Bionomics-EG&G 1975)
		grass shrimp [0.7](Tourat and Rao 1987)
		stream inverts [1] (Hansen and Garton 1982a)
		stoneflies and mayflies[1]
		    (Hansen and Garton 1982b[1])

>1 to 10	Ostracoda[3][2.5](Liber and O'Halloran 1995)	dipterans[10] (Hansen and Garton 1982a[1]) mixed insects[3] [1.9](Liber 1995) Ostracoda[3][7](Liber and O'Halloran 1995) mayflies, damselflies, and dragonflies[2.5] (O'Halloran and Liber 1995)
>10 to 100	coleoptera, oligochaetes, and gastropods[1][50] (Hansen and Garton 1982a) rotifers[3][30](Liber and O'Halloran 1995)	
>100 to 1000	clams [320](Surprenant 1989)	

[1] Macrocosm study
[2] No molting during short term exposures
[3] Litoral enclosures
[4] Marginal signs of toxicity
[5] Cited in U.S. EPA (1997a) as Beltsville Lab Test 2424. This study is not identified by MRID number or otherwise described.

Table 4-10: Summary of field studies on the effects of diflubenzuron on aquatic invertebrates [1]

Range of Application Rates (g/ha)	Species [conc ppb](Reference)		
	No Adverse Effects	**Adverse Effects with Observed Recovery**	**Adverse Effects with No Observed Recovery**
>0.1 to 1	pond invertebrates [0.2] Ali et al. 1988		
>1 to 10	shrimp, cyclops, and some cladocera (*Bosmina*), worms [3.7] (Ali and Mulla 1978a) worms [7.4] (Ali and Mulla 1978a)	zooplankton mortality and insect emergence [1.8](Wan and Wilson 1977) daphnids and copepods [3.7] (Ali and Mulla 1978a) copepods, shrimp [7.4] (Ali and Mulla 1978a) cladocera, copepods [2.5 to 10](Apperson et al. 1977) cladocerans, copepods and rotifers[10](Boyle et al. 1996)	amphipods [3.7] (Ali and Mulla 1978a) amphipods, daphnids [7.4] (Ali and Mulla 1978a)
>10 to 100	rotifers [13](Colwell and Schaefer 1980)	cladocera [10.4](Lahr et al. 2000) cladocera incl. *Bosmina*, copepods, [13](Colwell and Schaefer 1980)	shrimp [10.4](Lahr et al. 2000)

[1] The concentrations given in braces [] represent peak or typical concentrations shortly after exposure. In all cases, post-application concentrations will decline. See text for discussion.

Figure 3-1: Overview of the *In vivo* and environmental metabolism of diflubenzuron (adapted from WHO 1996).

Figure - 1

Figure 3-2: Modeled concentrations of diflubenzuron (thick lines) and 4-chloroaniline (thin lines) in ponds at an annual rainfall rate of 150 inches (see text for discussion).

Figure - 2

APPENDICES

Appendix 1:   Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Appendix 2:   Laboratory and simulation studies on environmental fate of diflubenzuron and its
                metabolites

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its
                formulations

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its
                formulations

Appendix 4:   Toxicity of diflubenzuron and diflubenzuron formulations to birds

Appendix 5:   Toxicity of diflubenzuron to terrestrial invertebrates

Appendix 6:   Toxicity of diflubenzuron to fish

Appendix 7:   Toxicity of diflubenzuron to aquatic invertebrates

Appendix 8:   Toxicity of diflubenzuron to aquatic plants

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Oral			

<div align="center">

Diflubenzuron

</div>

Acute Oral

Animal	Dose/Exposure	Response	Reference
Mouse and rat	LD $_{50}$, technical grade	> 4640 mg/kg	WHO 1996
Mouse and rat	LD $_{50}$,90% concentrate	> 5000 mg/kg	WHO 1996; U.S. EPA 1997a
Mouse and rat	LD $_{50}$, Du 112307 W.P. 25% (Dimilin WP 25%)	> 40,000 mg Dimilim/kg > 10,000 mg DFB/kg A marginal effects on methemoglobin levels.	Koopman 1977 MRID 00070025
Rat, Sprague-Dawley, 5 males(290-330 g) and 5 females (215-233 g), 9- to 12-weeks old	single gavage dose of 5000 mg/kg Dimilin 2L (22.36% pure)	No mortality. Except for moist rales in two treated rats on the day of dosing, no clinical signs of toxicity, all rats gained weight both 7 and 14 days after dosing, and no abnormalities observed during macroscopic postmortem evaluation. NOEC = 5000 mg/kg as Dimilin 2L 1118 mg/kg as DFB	Blaszcak 1997a MRID 44574504

Subchronic Oral

Animal	Dose/Exposure	Response	Reference
Cat (NOS)	0, 30, 100, 300, or 1000 mg/kg/day diflubenzuron for 3 weeks	NOEC (Hb) >1250 mg/kg/day NOEC (%PCV) not estimated NOEC (RBC) not estimated NOEC (reticulocyte count) not estimated NOEC (MetHb) = 30 mg/kg/day NOEC (SulpHb) = 3 mg/kg/day (calculated with regression analysis) NOEC (spleen weight) >1000 mg/kg/day	Keet et al. 1982

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Dogs, beagle, pure-bred, 15 males and 15 females	dietary levels of 10, 20, 40 or 60 ppm (**actual dosages levels of 0.42, 0.84, 1.64, or 6.24 mg/kg/day**) Du 112307 for 13 weeks	No mortality; no clinical signs of toxicity, no adverse effects on food or water consumption, no ocular effects, no treatment-related macroscopic post mortem findings, no adverse effects on organ weights, and no morphological abnormalities considered to be treatment related. At 2 weeks, all laboratory tests were within normal limits; at 4 and 6 weeks, SAP and SGPT were increased among some dogs at 40 or 160 ppm; after 6 weeks, the presence of **methaemoglobin** and other abnormal haemoglobin pigments was apparent in dogs at 160 ppm; after 12 weeks, one dog at 160 ppm had an elevated SGPT level and one dog at 160 ppm and one dog had a greater **methaemoglobin** value than all the other dogs. NOEC = 20 ppm	Chesterman et al. 1974 MRID 00038706
Dog (NOS)	0, 2, 10, 50, or 250 mg/kg/day diflubenzuron for 13 weeks	NOEC (Hb) = 10 mg/kg/day NOEC (%PCV) not estimated NOEC (RBC) >250 mg/kg/day NOEC (reticulocytes) = 50 mg/kg/day NOEC (MetHb) = 50 mg/kg/day NOEC (SulpHb) = 10 mg/kg/day NOEC (spleen weight) not estimated	Keet et al. 1982

Appendix 1-2

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Mice, 40/sex/dose group	in diet concentrations of 0, 80, 400, 2000, 10,000, or 50,000 ppm 97.2% pure, technical grade, air-milled diflubenzuron for 14 weeks with 7-week interim sacrifice. The **calculated mean intake** of diflubenzuron was 9.7, 50.7, 240, 1174, or 6114 mg/kg/day (males) and 11.1, 54.9, 288, 1393, or 7506 mg/kg/day (females) [*cf* page 27]	No treatment-related mortality throughout the study; no significant, treatment-related changes in food consumption or body weight; numerous hematological effects, including statistically significant increases (see pg 29) in Met Hb% and Sulph Hb% in males and females at 400-50,000 ppm; statistically significant increase in spleen weight in males and females at 400-50,000 ppm; statistically significant increase in liver weight of males and females at 2000-50,000 ppm;	Colley et al. 1981 MRID 00114330
Rats, Swiss-albino, males, weighing 90 g, 5/dose group	gavage doses of 96.7 mg/kg of Dimilin in corn oil solution each day for 48 days (i.e., total of 4640 mg/kg of Dimilin)	Mean hemoglobin concentration (g/100 mL blood) was significantly lower than that of controls; mean hematocrit percent of the Dimilin was significantly higher than that of controls.	Berberian and Enan 1989
Rats, Sprague-Dawley, 40/sex/ dose group	in diet concentrations of 160, 400, 2000, 10,000, or 50,000 ppm technical grade diflubenzuron for 90 days	No mortality; no clinical signs of toxicity, no adverse effects on body weight or food consumption. Treatment-related adverse effects included a significant increase in **methemoglobin** at weeks 7 and 13 in males at 400, 2000, 10,000, and 50,000 ppm and in females at all dose levels, as well as significant increases in sulfhemoglobin at week 7 in 50,000 ppm males and 10,000 and 50,000 ppm females, and at week 13 in males at 10,000 and 50,000 ppm and in females at 2000, 10,000, and 50,000 ppm. Other pathological, treatment-related changes included decreases in hematocrit and hemoglobin values and the erythrocyte count and an increase in the number of reticulocytes, increases in absolute liver weight and absolute and relative spleen weights, and enlargement of the spleen. NOEC (for males only) = 160 ppm	Burdock et al. 1980 MRID 00064550

Appendix 1-3

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Sheep (NOS)	0, 25, 125, or 500 mg/kg/day diflubenzuron for 13 weeks.	NOEC (Hb) >500 mg/kg/day NOEC (%PCV) >500 mg/kg/day NOEC (RBC) >500 mg/kg/day NOEC (reticulocyte count) not estimated NOEC (MetHb) = 25 mg/kg/day NOEC (SulpHb) = 3 mg/kg/day (calculated with regression analysis) NOEC (spleen weight) >500 mg/kg/day	Keet et al. 1982
Sheep	0, 500, 2500 and 10,000 mg/kg in feed for 13 weeks.	No treatment-related effects were observed on food consumption, body weight gain, hematological parameters or urinalysis. Increase in MetHb and SulfHb and a reduction in the weight of the thyroid.	Ross et al. 1977

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Chronic Oral			
Dogs, beagle, 5/sex/dose group	daily oral administration of 0, 2, 10, 50, or 250 mg/kg/bw technical grade, air-milled diflubenzuron via gelatin capsules, 7 days/week for 52 consecutive weeks.	There were no clinical signs of toxicity, no treatment-related effects on body weight, food consumption, or water consumption; no ocular effects; there were treatment-related *marginal but statistically significant* increases in met Hb% and sulph Hb% (at ≥10 mg/kg/day bw) and in Heinz body counts (at 50 and 250 mg/kg/day bw); there was a marginal but consistent compound-related decrease in MCHC (at ≥10 mg/kg/day bw); histopathological changes included increased spleen weight (statistically significant in males at ≥50 mg/kg/day bw), increased liver weight (significant at ≥50 mg/kg/day bw in males and females) and hemosiderin deposition in the liver. The investigators conclude: *the no effect level demonstrated...was 2 mg/kg/day. However, this level is based on minor hematological changes of no toxicological significance seen at 10 mg/kg/day. Hence it is more realistic to consider the no effect level based on organ weights and histopathology as being at least 10 mg/kg/day.* Mortality: 2 females dogs died during the study. One dog at 250 mg/kg/bw) was sacrifice *in extremis* at week 33 due to liver failure and the other dog (at 50 mg/kg/day bw) died during week 40 due to bronchopneumonia. These effects were not attributable to treatment.	Greenough et al. 1985 MRID 00146174 **[This study is the basis for the chronic RfD]**

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Mice, CFLP, approximately 8 weeks old, 36/sex/dose group	In diet concentrations of 16, 80, 400, 2000, or 10,000 ppm (**intake values = 1.24, 6.40, 32. 16, 163.29, or 835.55 mg/kg/day for males and 1.44, 7.26, 35.38, 186.59, or 958.51 mg/kg/day for females**) technical grade DFB (97.6% pure) for 91 weeks.	Treatment-related clinical sign of toxicity was a blue/gray discoloration of the extremities and dark eyes in all mice at 10,000 ppm, a majority of mice at 2000 or 400 ppm, and in a number of mice at 80 ppm. The NOEC for this effect =16 ppm. No obvious treatment-related effect on mortality was observe; no obvious treatment-related effect on food consumption, body weight, food efficiency, or water intake was observed; treatment-related changes were principally associated with oxidation of the haemoglobin or with hepatocyte changes. DFB is not carcinogenic to DFLP mice.	Keet et al. 1984b
Mice, 88/sex/dose group	in diet concentrations of 0, 16, 80, 400, 2000, or 10, 000 ppm 97.6% pure difllubenzuron for 91 weeks. The **calculated mean intake** of diflubenzuron was 1.24, 6.40, 32.16, 163.29, or 835.55 mg/kg/day (males) and 1.44, 7.26, 35.38, 186.59, or 958.51 mg/kg/day (females) [*cf* page 47]	No treatment-related mortality throughout the study, no evidence of tumorigenic effect; treatment-related effects were primarily associated with oxidation of haemoglobin (**treatment-related increases in Met Hb% were recorded from week 26 onwards and in Sulph Hb% from week 52 onwards; these changes principally affected mice at 80-10,000 ppm and were dose-related in degree**) or with hepatocyte changes (**an increased incidenc of hepatocyte enlargement was observed in males and females at 400-10,000 ppm**).	Colley et al. 1984 MRID 00142490
Rats, Sprague-Dawley, 50/sex/dose group	in diet concentrations of 0, 156, 625, 2500, or 10,000 ppm technical grade diflubenzuron (97.6% a.i.) for 104 weeks.	No treatment related effects with regard to mortality or clinical observations; no evidence of carcinogenicity after 2 years of dietary exposure to diflubenzuron; statistically significant dose-related increases in met Hb% and sulph Hb% in males and females; numerous hematological effects; histomorphological changes observed in sections of the spleen, liver, and bone marrow; in general adverse effects were most pronounced at the 2500 and 10,000 dose levels.	Burdock 1984 MRID 00145467

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Rats, Sprague-Dawley, approximately 7 weeks old, 50/sex/dose group	In diet concentrations of 156, 625, 2500, or 10,000 ppm **(intake values =6.99, 28, 36, 114.35 or 463.80 mg/kg/day for males and 9.23, 37.98, 153.96, or 633.41 mg/kg/day for females**) technical grade DFB (97.6% pure) for 104 weeks.	No treatment related clinical signs observed; no obvious treatment-related effect on mortality; no obvious treatment-related effect on food consumption or body weight, except in high dose females where terminal body weight was significantly less than controls; no evidence of tumorigenic effects, treatment-related changes were principally associated with oxidation of haemoglibin or with hepatocyte changes. DFB is not carcinogenic to Sprague-Dawley CR-CD rats.	Keet et al. 1984a

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference

Reproduction Studies

Animal	Dose/Exposure	Response	Reference
Rats, Crl:CD(SD)BR	0 or 1000 mg/kg bw per day on days 6–15 of gestation	Screening assay for teratogenicity. No signs of developmental toxicity, birth defects or maternal toxicity.	Kavanagh 1988a
Rabbits, New Zealand White	0 or 1000 mg/kg bw per day on days 7–19 of gestation	Screening assay for teratogenicity. No signs of developmental toxicity, birth defects or maternal toxicity.	Kavanagh 1988b
Rats, Charles River 32/sex/dose group	in diet nominal concentrations of 0, 500, 5000, or 50,000 ppm technical diflubenzuron through two consecutive generations. F_0 generation mean intake values (weeks 1-10 *pre-mate*) were 36.2, 360, or 3755 mg/kg/day for males and 42.0, 427, or 4254 mg/kg/day for females. F_1 generation mean intake values (weeks 5-16 *pre-mate*) were 39.2, 394, or 4089 mg/kg/day for males and 44.9, 473, or 4611 mg/kg/day for females	No treatment-related morality; toxicity manifested as hematological effects characterized primarily by anemia and increases in MetHb% associated with increased spleen weight and pathological lesions of hemosiderosis of the spleen and brown pigmented Kupffer cells in the liver were observed all dose levels. Increases in MetHb ranged from about 115% in the low dose group to over 300% in the high dose group (see Section 3.3 for more complete discussion and details). Other treatment related effects on the parental rats included lower body weight gains of the F_0 generation at 50,000 ppm, with higher food intake values in males; increased water consumption among males and females at 5000 or 50,000 ppm and among males at 500 ppm. No treatment-related effects on reproductive performance at any dose level. In the F_1 generation, liter and mean pup weights of the offspring from parents in the 50,000 dose group were lower than controls. The effect was not observed in the F_2 offspring. NOEL = 50,000 ppm for reproductive function NOEL = 5000 ppm for pre-weaning development of the offspring. NOEL = >500 ppm for MetHb	Brooker 1995 MRID 43578301 NOTE: U.S. EPA (1996) appears to classify the low dose group as the LEL for MetHb but specifies the dose as 25 mg/kg/day. This error appears to be based on the use of default values for converting food concentrations to mg/kg/day doses.

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
DERMAL			
Rabbits, New Zealand white, 5 males and 5 females	Dermal application of 5000 mg/kg Dimilin 2L (22.36% pure) to closely clipped intact trunks (approximately 10% of the body surface area). Treated area covered with gauze and occlusive wrap for 24 hours.	No mortality; no pharmacological or toxicological signs of toxicity; no severe dermal effects; no abnormalities observed during postmortem macroscopic evaluation. NOEC = 5000 mg/kg	Blaszcak 1997b MRID 44574505
Rabbits, New Zealand white, 4 males and 2 females, young adults, 2.2-2.6 g	Dermal application of 0.5 mL Dimilin 2L (22.36% pure) to intact skin of backs (hair closely clipped). Test site was semi-occluded with gauze for 4 hours	4/6 rabbits had slight, barely perceptible, erythema; 1/6 had slight erythema; 1/6 had no signs of dermal irritation. Dimilin 2L considered *slightly irritating* (FIFRA Primary Irritation Index = 0.5)	Blaszcak 1997d MRID 44574508
Guinea pigs, Dunken Hartley, 10/sex	Induction dose of approximately 0.3 mL Dimilin 2L (22.36% pure) for 6 hours; challenge dose after 14 days with 100% test material	No dermal sensitization responses during induction or challenge phase.	Blaszcak 1997e MRID 44574509
Rats. Charles River, 10/sex/dose group, weight = 284-314 g (males) and 201-233 g (females)	Dermal application of 20, 500, or 1000 mg/kg/day Dimilin (technical diflubenzuron) to shaved intact skin for 21 days.	No treatment-related effects on survival, clinical signs of toxicity, dermal observations, body weights, food consumption or macroscopic and microscopic pathology. Females in the 500 and 1000 mg/kg/day group had mild but statistically significant decreases in mean erythrocyte counts, hemoglobin, and hematocrit values; males in the 1000 mg/kg/day group had mild but statistically significant decreases in mean hemoglboin and hematocrit values. At 500 and 1000 mg/kg/day, males and females had an increased incidence of polychromasia, hypochromasia, and anisocytosis. At 1000 mg/kg/day, males and females had mild but statistically significant increases in Met Hb values and males also had mildly increased Sulph Hb values. NOEL = 20 mg/kg/day.	Goldenthal 1996 MRID 43954100-01

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Rats, Sprague-Dawley, males, 12/dose group	single dermal applications of ^{14}C-diflubenzuron suspended in 0.25% (w/v) gum tragacan WLC-grade water at 0.005 or 0.05 mg/cm^2 to shaved skin for periods of 1, 4, and 10 hours.	> 89% of the applied dose was removed by washing; 6% of the applied dose was found in the skin and increased exposure time did not increase the percent of dose found in the skin, although the amount of test material found in the skin was nearly proportional to dose; blood, carcass, and excreta accounted for negligible amounts of the applied dose; systemic absorption, excluding the skin was <1% of the total applied dose. These data indicate that the material that was absorbed was absorbed quickly. and the percent of applied dose that was absorbed appeared to be constant regardless of dose.	Andre 1996 MRID 44053101

EYES

Animal	Dose/Exposure	Response	Reference
Rabbits, New Zealand white, 6	0.1 mL Dimilin 2L instilled in lower conjunctival sac of the right eye of each rabbit. Observations for ocular irritation made at 1, 24, 48, and 72 hours.	Positive scores (slight to moderate conjunctival irritation) in 3/6 rabbits within 24 hours of exposure with full recovery within 48 hours. No signs of iridial or corneal changes. The remaining 3 rabbits did not have positive scores for ocular irritation at any time during the study. Study demonstrates that Dimilin 2L is an "eye irritant" based on the results of positive scores in 3/6 animals with all changes being reversible.	Blaszcak 1997c MRID 44574507

INHALATION

Animal	Dose/Exposure	Response	Reference
Rats, Sprague-Dawley, approximately 6-weeks old, 10/sex/dose group	Nose-only exposure to 0, 10, 30, or 100 mg/m^3 Dimilin technical 6 hours/day, 5 days/week for 4 consecutive weeks.	Dimilin technical produced minimal toxicity, including a slight (5-7%) decrease in erythrocytes, slight statistically significant decreases in hemoglobin and hematocrit in males and females at 100 mg/m^3 and an increase in bilirubin in males at 100 mg/m^3. No treatment-related effect observed on methemoglobin. NOEC = 30 mg/m^3	Eyal 1999 MRID 44950601

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Rats, Sprague-Dawley, 9 weeks old, 5 males (323-335 g) and 5 females (234-249 g)	4-hour nose only exposure to 2.0 mg/L Dimilin 2 L (22.36% pure) with 14-day post exposure observation period	No mortality; signs of toxicity during exposure included red nasal discharge and labored breathing; chromodacryorrhea, red nasal discharge, and excessive salivation, labored breathing, and moist rales were observed in some rats up to 1 day after exposure with complete recovery thereafter; slight weight loss was observed in some females during the first week after exposure followed by complete recovery during the second week; no abnormal macroscopic effects were observed during postmortem evaluation. LC_{50} >2.0 mg/L	Hoffman 1997 MRID 44574506
Rats, Sprague-Dawley, 20 males and 20 females, 5/sex/dose group	Whole body exposure to nominal concentrations of 0.5, 5.0, or 50 mg/L air 5 days/week for 3 weeks. Corresponds to 500, 5000, and 50,000 mg/m^3 – i.e., 1000 L = 1 m^3.	No signs of irritation at 0.5 mg/L; frequent blinking and occasional bouts of persistent sneezing and slightly labored breathing during exposures to 5.0 mg/L, followed by rapid recovery between exposures; at 50 mg/L, the signs observed in the mid-dose group were more pronounced and more persistent but repeated exposure did not result in cumulative adverse effects and recovery was rapid after each exposure period. No changes in body weight, compared with controls and no effects on water or food consumption were observed. Post-exposure **methaemoglobin** levels were increased 0.2-0.5 g% over controls (0.1 g%). The increase was statistically significant in the mid and high-dose males and in all treated females.	Berczy et al. 1975 MRID 00044325

Appendix 1-11

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Rats, Sprague-Dawley, males and females, 6-weeks-old, 10/sex/dose group	Nose-only exposure to 0, 10, 30, or 100 mg/m^3 (measured as 12, 34, or 109 mg/m^3 diflubenzuron technical (95.6% purity) 6 hours/day, 5 days/week for 4 weeks.	Minimal toxicity: slight decrease (5-7%) in erythrocytes, slight statistically significant decreases in hemoglobin and hematocrit in males and females at 100 mg/m^3; increase in bilirubin males at 100 mg/m^3. A reduction in 'grid count' was evident in a neuro-functional assessment at the highest concentration. No effect observed on methemoglobin. NOEC = 30 mg/m^3	Newton 1999 MRID 44950601

4-chloroaniline

Animal	Dose/Exposure	Response	Reference
Rats, Wistar, SPF albino, males and females, 10/sex/dose group	daily oral doses of 0, 8.0, 20.0, or 50.0 mg/kg **4-chloroaniline (4-CA)** for 3 months	All rats at 50 mg/kg had increased numbers of Heinz bodies (>20/100 RBC) and a reticulocyte response (>2%); however there was no evidence of a decrease in hemoglobin, packed cell volume, or RBC count. Histological changes were observed only in the high dose group and included increased extramedullary haematopoiesis in spleen and liver and occasionally in the lung; increased hemosiderin (from hemoglobin breakdown) in the liver and spleen and occasionally in the kidneys (epithelium of proximal convoluted tubules). NOEC = 8.0 mg/kg	Scott and Eccleston 1967

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Dog, Beagle, males and females, 4/sex/dose group	daily oral doses of 0, 5, 10, or 15 mg/kg **4-chloroaniline (4-CA)** for 3 months	One dog in the 15 mg/kg dose group died as a result of excessive haemolysis (after receiving 25 mg/kg 4-CA). 5/7 remaining dogs receiving 15 mg/kg 4-CA showed an early and marked decrease in RBC count (>1.5 M) and packed cell volume (>15%) with a concomitant decrease in hemoglobin levels. The same trend was observed in half the dogs at 10 mg/kg and one of the dogs at 5 mg/kg. Lowest levels of RBC and hemoglobin were reached at approx 3-4 weeks, after which time, there was a slow but steady improvement in all values, despite the persistence of increased numbers of Heinz bodies. A reticulocyte response and an in crease in Heinz bodies were observed in all dogs at 15 mg/kg, most dogs at 10 mg/kg, and three dogs at 5 mg/kg, while the control group remained normal. All treated dogs showed histological changes, including evidence of hematopoietic response in extramedullary activity in spleen and liver at all doses (The marrows showed hyperplasia of the erythroid phase) and marked evidence of RBC destruction in the spleen, and liver.	Scott and Eccleston 1967
Rats, Fischer 344, males, 10/dose group	In diet concentrations of 1240 ppm **4-chloroaniline** or 1240 or 4320 ppm **p-chlorophenylurea** for 7 days	1240 ppm **4-chloroaniline** caused statistically significant increases in methemoglobin values at all intervals of analysis No treatment related effects on methmoglobin values in rats treated with 1240 or 4320 ppm **p-chlorophenylurea**. The only macroscopic change observed was enlargement of the spleen in rats from the 1240 ppm **4-chloroaniline** group. No mortality.	Goldenthal 1999b MRID 44871303

Appendix 1: Toxicity of diflubenzuron and 4-chloroaniline to experimental mammals

Animal	Dose/Exposure	Response	Reference
Rats, Fischer 344, approx 6-weeks old, males and females, 25/sex/dose group	In diet concentrations of 250 or 500 ppm **4-chloroaniline** for 78 weeks with a 24-week observation period.	Mean body weight depression associated with treatment was observed in high dose females, compared with controls. No significant treatment-related mortality among females; however, there was a significant (p=0.0294) correlation between dose and mortality in males rats. In the high dose male rates, the incidence of unusual splenic neoplasms (i.e., fibroma, fibrosarcoma, sarcoma, hemangiosarcoma, and osteosarcoma) was increased (0/20 controls; 0/49 low dose, 10/49 high dose). This finding was considered strongly suggestive of carcinogenicity because of the rarity of the rumors in the spleens of controls rats. Formation of non-neoplastic lesions of the splenic capsule in rats in all dose groups.	NCI 1979
Mice B63CF1, approx 6-weeks-old, males and females, 25/sex/dose group	In diet concentrations of 2500 or 5000 ppm **4-chloroaniline** for 78 weeks with a 24-week observation period	Mean body weight depression associated with treatment was observed in all mice, compared with controls. No significant treatment-related mortality in mice of either sex.	NCI 1979

Appendix 2: Laboratory and simulation studies on environmental fate of diflubenzuron and its metabolites.

Data Summary	Reference

Aquatic Sediments

anaerobic aquatic metabolism of 1.3 mg/kg ^{14}-C diflubenzuron in silt loam/water system.

Thus et al. 1991
MRID 41837601

DT_{50} = 34 days for total hydrosoil/water system and 18 days for water-phase only.

2,6-difluorobenzoic acid, and 4-chlorophenylurea were main metabolites that accumulated in the anaerobic water phase; hardly any bound residue detected.

two model ditch (water/sediment) systems (sandy loam or silt loam covered with surface water) with addition of 0.94 ppm. diflubenzuron with continuous flow through upper layer of surface water. Incubation at 20±1° w/12 hour photo period.

Thus and van der Laan-Straathof 1994
MRID 44399307

Results indicate rapid disappearance of compound from model ditch systems due to rapid metabolism and adsorption to sediment.

Water phase DT_{50} = 1.1 day (silt loam) and 1.9 days (sandy loam).
Complete sediment/water systems DT_{50} = 10 days (silt loam/surface) and 25 days (sandy loam/surface).

Only metabolites were DFBA and CPU

0.013 ppm DFB in a microbially viable soil/water test system

Dzialo and Maynard 1999
MRID 44895001

DFB was readily degradable under aerobic aquatic conditions
half-life (first-order kinetics) = 25.7 days (r^2=0.709)
DT_{50} = 5.3 days

major metabolite formed, 4-chlorophenylurea
half-life (first-order kinetic) = 39.7 days (r^2=0.671)

degradation rate of 50 µg/L diflubenzuron in seawater in the presence of salmon feces and sediment is temperature dependent: at 15°, DT_{50} = 3 ½ weeks (anaerobic) or 4 ½ weeks (aerobic); however at 5°C, there was no significant difference between the anaerobic (DT_{50} = 99 days) or the aerobic (DT_{50} = 100 days) test conditions.

van der Laan 1995
In: Technology Sciences Group 1998
MRID 44399307

The metabolites included 4-chlorophenylurea, 2,6-difluorobenzoic acid, 2,6-difluorobenzamide, and CO_2

Appendix 2: Laboratory and simulation studies on environmental fate of diflubenzuron and its metabolites.

Data Summary	Reference
laboratory microcosm study using 10 µg/L DFB in seawater with or without sediment.	Wilson et al. 1995

half-life of DFB in seawater **without** sediment = 18.7 days
half-life of DFB in seawater **with** sediment = 5.2 days

presence of organic sediment in DFB-treated microcosm significantly reduced the efficacy of DFB in seawater as measured by toxicity of aged DFB (initial nominal concentration of 10 µg/L) to 5-day old grass shrimp embryos. By day 30, embryos reared in seawater from DFB-sediment microcosm produced larvae with no signficant morphological abnormality and larval viability was comparable to controls; embryos reared in DFB-treated seawater **without sediment** produced larvae with severe abnormalities and very low viability even after the seawater aged for 65 days.

persistence of diflubenzuron (Dimilin) in sod-lined water pools after repeated applications:	Madder and Lockhart 1980

Bioassay data indicate toxicity greatest during the first 24 hours; DFB fell below detection limits (1µg/L) within 24 hours, whereas chlorophenylurea concentration increased for several days after treatment.

Bioconcentration

Channel catfish, *Ictolurus punctatus*: No bioconcentration. In 0.01 ppm tanks, concentration in muscle was below 0.002 ppm and concentration in viscera peaked at about 0.003 ppm (Figure 2). Similar pattern in 0.5 ppm tanks (Figure 3).	Booth and Ferrell 1977

Algae: BCFs of 2412 at hour 1 to 109 at day 4. Probably reflects degradation – i.e., algae degraded 80% of the DFB in a 1-hour incubation period.

Laboratory algae culture system of *Scenedesmus subspicatus* exposed at an initial concentration of 200 µg/L DFB for 7 days	Yu-Yn et al. 1993

no growth inhibition; half-life = 3 days

DFB radioactivity in algae increased steadily and leveled off at approx. 60% after 5 days

BCF values decreased from 4310 to 889 during the exposure period

elimination was rapid during the first hours.

Hydrolysis

rapid decrease in of residue levels.	Anton et al. 1993

Half-life w/aeration = 0.41899 days (tap water and natural sunlight)
Half-life wo/aeration = 0.96685 days (tap water and natural sunlight)

Appendix 2: Laboratory and simulation studies on environmental fate of diflubenzuron and its metabolites.

Data Summary	Reference
Two applications of Dimilin 25W (25% a.i. by weight) to surface of littoral enclosures using portable hand sprayer at rates of 4-210 g/ha.	Knuth 1995 MRID 44386201 (This is chapter 2 of Moffet 1995)
Maximum residues in water column measured withing first 24 hours after application,	
Half-lives ranged from 3.28 to 8.23 days with a mean of 4.28 days. By 14-35 days (or a mean of 18.5 days), 95% of the diflubenzuron dissipated. Principal loss from water column early in the study probably due to adsorptive processes because temperature and pH were not favorable for rapid aqueous hydrolysis.	
11 µg/L ^{14}C-diflubenzuron in a CO_2-evolution test (concentration below aqueous solubility).	van der Laan and Thus 1993 *In*: Technology Sciences Group 1998 MRID 44399307
DT_{50} = approximately 2.5 days; hydrolysis products are DFBA, CPU, and CO_2	
High temperature (121˚C) increased the degradation of diflubenzuron in aqueous media at levels greatly above its solubility in water and resulted in its rapid degradation to as many as seven identified products: 4-CPU, 2,6-DFBA, 2,6-difluorobenzamide, 4-chloroaniline, *N,N'*-bis (4-chlorophenyl) urea, 1-(4-chlorophenyl)- 5-fluoro-2,4 (1H,3H)-quinazolinedione and 2-[(4-chlorophenyl) amino]- 6-fluorobenzoic acid.	Ivie et al. 1980
4-Chloroaniline, *N,N'*-bis (4-chlorophenyl) urea and 2[(4-chlorophenyl) amino]-6-fluorobenzoic acid were not detected at lower temperatures (0.1 mg [^{14}C]-diflubenzuron/L water or buffer at 36˚C). 4-Chloroaniline was a major degradation product of diflubenzuron in heat-treated samples, but it was not seen at lower temperatures	

Photolysis

Photodegradation half-lives of diflubenzuron in deionized water (pH 7) = 17 hours; in deionized water (pH 9) = 8 hours; and in river water (pH 9) = 12.3 hours.	Marsella et al. 2000
In a solar simulator using river water buffered to pH 9.0, the half-life for diflubenzuron =12 hours; dark controls showed no loss of parent compound over similar time periods.	
Log Kow = 3.8 (determined using reverse phase HPLC)	

Data Summary	Reference

Residues on Plants

persistence of diflubenzuron (commercial grade 25% WP) on Appalachian forest leaves.

Harrahy et al. 1993

Leaves sprayed in spring and left to weather during growing season.

white oak leaves collected in July and August and placed in headwater stream to monitor residual diflubenzuron showed rapid decrease in residue (36% in July and 23% in August) within the first 48 hours of stream incubation, reaching less than 10% of the original concentration within 3 weeks.

Yellow poplar, read maple, and white oak leaves collected in December and place in headwater stream showed a much slower rate of loss. After 54 days in the stream, yellow poplar and red maple leaves retained 45 and 40%, respectively of the original diflubenzuron concentration and white oak showed no significant loss.

Stream water temperatures averaged 17°C lower in December than in August (temperature readings were not made in July).

Soil Degradation/Transport

fate of 4-chloroaniline in nonautoclaved and autoclaved soil.
in soil treated with 4-chloroanline and incubated for 6 weeks, no CO_2 evolution in occurred in autoclaved soil; in nonautoclaved samples, CO_2 was determined as 7.5% of the originally applied radioactivity.

Bollag et al. 1978

Cell suspension of 0.04 g *Pseudomonas putida* in 2 mL of 0.05 *M* phosphate buffer (pH 7.0) incubated with 10 µg ^{14}C-PH-6040 (DFB) (both A and B labels) for 6 hours produced no evidence of degradation upon extraction. Both labeled preparation were recovered intact as 99.9+% of total ^{14}C

Metcalf et al. 1975

10 ppm ^{14}C-PH-6040 (DFB) added to fresh, air-dried Drummer soil (17.4% moisture) and incubated at 80°F for 1, 2, or 4 weeks.

Metcalf et al. 1975

Compound appeared to be very stable, with degradation products comprising only 0.7% of total extracted radioactivity after 4 weeks.

aerobic soil metabolism of 0.69 mg/kg ^{14}-C diflubenzuron in sandy loam

Walstra and Joustra 1990
MRID 41722801

$DT_{50} = 50$ hours; $DT_{90} = 181$ hours

CO_2, 2,6-difluorobenzoic acid, and 4-chlorophenylurea were main metabolites; 2,6-difluorobenzamide and 4-chloroaniline were minor metabolites.

Appendix 2: Laboratory and simulation studies on environmental fate of diflubenzuron and its metabolites.

Data Summary	Reference
10 ppm technical DFB applied on quartz sand to natural sandy loam and much soils at 12 weeks: 2-12% remaining (compared with 80-87% remaining in sterilized soil), indicating that soil microorganisms play a major role in the degradation of DFB. Kinetic analysis based on 1st order dependence indicates that the rate constants for disappearance reactions decreased with time.	Chapman et al. 1985
breakdown of DFB by soil isolates: *Rhodotorula* sp. half-life of detectable DFB = 18 days (carbon source: acetone) *Fusarium* sp. half-life of detectable DFB = 7 days (carbon source: DFB/acetone) *Penicillium* sp.half-life of detectable DFB = 14 days (carbon source: acetone) *Cephalosporium* sp.half-life of detectable DFB = 13 days (carbon source: acetone) Control half-life of detectable DFB = 27 days.	Seuffer et al. 1979
^{14}C-DFB readily degraded in various agricultural soils and in hydrosoil: 50% of applied dose (1 mg/kg) metabolized in ≤2 days. Chief products of hydrolysis were 4-chlorophenylurea and 2,6-difluorobenzoic acid.	Nimmo et al. 1984
initial dose of 1 mg/kg **4-chlorophenylurea** in decreased to 50% in about 5 weeks in aerobic sandy clay and in about 16 weeks in anaerobic hydrosoil Investigators assume that two sorts of bound residues are formed from **4-chloro-phenylurea**: one is fairly stable and might consist of bound 4-chloroaniline or its transformation products and the other is presumed to be a degradable derivatie of **4-chlorophenylurea**.	Nimmo et al. 1986
2-6-difluorobenzoic acid is rapidly and completely degraded in soil: time to 50% disappearance in 9 days in humus sand and after 12 days in sandy clay. DFBA completely disappeared in the humus sand after 32 days.	Nimmo et al. 1990
DFB (technical), Dimilin WP-25, and Dimilin SC-48 were applied separately at 70, 210, or 630 g ai./ha (corresponding to 17.23, 51.69, or 155.07 µg a.i.) To top layer of columns (30x5.6 cm id) packed either with sandy or clay loam forest soils. Mobility of DFB was low and did not increase with dosage. At deposit rate equivalent to 70 g a.i./ha, nearly all the residues were found with 2.5 cm of the top of the column. At 630 g a.i./ha, only about 9% of the technical DFB, 7% of Dimilin SC-48, and 4% of Dimilin WP-25 moved below the 2.5 cm level in sandy loam. No residues were found below the 10 cm level or in the leachates in either soil type at all dosage levels. In addition to soil type, mobility of DFB was also influenced by the additives present in the formulations with technical DFB > Dimilin SC-48 > Dimilin WP-25.	Sundaram and Nott 1989

Appendix 2: Laboratory and simulation studies on environmental fate of diflubenzuron and its metabolites.

Data Summary	Reference
Organic soil and silty clay loam soil collected from a boreal forest in northern Ontario, Canada	Sundaram et al. 1997

maximum amount adsorbed: 88 μg/g (organic soil); 73 μg/g (silty clay loam)
time required for maximum adsorption: 18 h (organic soil); 24 h (silty clay loam)

Organic soil characterized as about equal parts sand, silt, and clay and 21% OM and 13% OC.

Silty clay loam characterized as 22% sand, 49% silt, and 29% clay, and 8.2% OM and 5.1% OC.

K_D = 17.59 (organic soil)
K_D = 16.42 (silty clay loam)

K_{oc} = 135.3 (organic soil)
K_{oc} = 332.0 (silty clay loam)

calculated K_{oc} = 144.4 (organic soil)
calculated K_{oc} = 345.3 (silty clay loam)

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
33, 66, and 140 g a.i./ha (0.5, 1, and 2 oz a.i./ac)	No evidence of negative effects on predators/parasites – lacewing (*Chrysopa carnea*), ladybird beetle (*Hippodamia convergens*), Wasp parasite *Trichograma pretiosum* of bollworm (*Heliothis*). Immigration from untreated fields could mask negative effects on beetles seen in lab (see Appendix 5).	Ables et al. 1977
280 g a.i./ha (4 oz a.i./ac)	Caged lacewing suffered increased mortal. eating treated eggs. No effect on parasitic wasp through 2-3 generations; wasp developed in treated eggs and in eggs produced by treated adults, and direct exposure to adults was not toxic.	Ables et al. 1977
187 ppm spray to apple orchards (NOS)	No adverse effects in Phytoseiid and stigmaeid mites No population increases following treatment in European red and rust mites	Anderson and Elliott1982
Application (spray) of Dimilin WP, 0.6 kg in 600 L/ha to a 2.4 ha apple orchard (integrated pest management program). 250 g Dimilin/ha, 62.5 g a.i./he	DFB persisted on foliage until leaf-fall and was detected on the peel of harvested fruit. Mean residue on harvested Worcester fruit = 0.05 mg/kg fresh weight and on harvested Cox fruit, mean residue = 0.02 mg/kg fresh weight.	Austin and Carter 1986
4-year field study (1992-1995) in apple orchards in a codling moth control program based on 4 seasonal sprays/year. Diflubenzuron at 3-12 g/100 L. Application rate in g/ha not specified.	Spider fauna (26 genera and 30 identifiable spider species) in apple orchards of Western Oregon. DFB was harmless to spider species tested (p>0.05)	Bajwa and AliNiazee 2001
Dimilin 4 liquid applied at 70 g a.i./ha to watersheds in a central Appalachian broadleaf forest	Yellowjackets, (10 species of wasps, Family Vespidae): Diflubenzuron decreased worker number during application year but not in post application year. There was no effect of trap site on worker sample size.	Barrows et al. 1994
140 g a.i./ha (2 oz a.i./ac). 4.05 ha in 41 ha woods.	Some species of soil mites were adversely affected. Half the number in treated v. untreated samples.	Blumberg 1986
67 g a.i./ha (0.96 oz a.i./ac)	Wasp parasite on Gypsy moth eggs (*Ooencyrtus kuvanae*) on gypsy moth. Egg masses in treated plots were parasitized as heavily as egg masses in control plots. Lab data showed no effect on development and emergence from treated eggs or from eggs laid by treated adults.	Brown and Respicio 1981

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
350 g a.i./ha (5 oz a.i./ac)	5 hives (Honey bee, *Apis mellifera*.) in treated and untreated sites. No effects on egg hatch, brood production, numbers of adults, and honey production.	Buckner et al. 1975
70 g a.i./ha (1 oz a.i./ac)	Under tree bands, Carabidae (beetles), Gryllacrididae (grasshoppers), and two families of moths were significantly reduced in total taxa richness and abundance on treated sites.	Butler 1993

Additional Notes on Butler 1993: Foliage sampling found reduced abundance and richness in the following groups: Lepidoptera, Symphyta (sawflies, horntails), some herbivorous Coleoptera (beetles), Psocoptera (book lice, wood lice), predatory Thysanoptera (thrips), some Homoptera (leaf hoppers, aphids, cicadas), Diptera (flies), Orthoptera (grasshoppers), and Arachnida (spiders). Some affected by direct toxicity and others (predators) indirectly through prey reduction.

Aerial application of Dimilin 4L (35.1 g a.i./ha) to two watersheds in a Central Appalachian forest; two untreated watersheds served as controls.	Gypsy moth larvae decreased in number on the treated watersheds, especially during the treatment and post-treatment year. Macro lepidoptera larvae also decreased in number during the treatment year.	Butler et al. 1997 Butler 1995

Additional Notes on Butler et al. 1997: In treated watersheds, there was an overall reduction in arthropod family diversity and abundance on foliage and a significant reduction in the number of macro Lepidoptera and beetles. 27 months after treatment, total arthropod abundance and macro lepidoptera abundance on foliage remained significantly reduced. Decreases in the numbers of Carabidae (ground beetles), Gryllacrididae (crickets), Psocoptera (booklice/barklice), Phlaeothripidae (alligatorweed thrips), and some sapfeeders were observed but reductions were not significant.

Aerial application of 0.0084, 0.0168, or 0.0336 kg a.i./ha Dimlin 2F [8.4, 16.8, 33.6 g/ha] or 0.0168 kg a.i./ha Dimilin 25W [16.8 g/ha] to mixed-grass rangelands near Amidon, ND (experimental plots were 0.4x0.4 km).	Abundance of ants was not significantly reduced by treatment at any levels. Ant diversity declined temporarily (13-19 days) after treatment with Dimilin 25W, but recovered immediately the following week and no further declines were observed. Twenty species of ants were encountered in the experimental site.	Catangui et al. 1996
Aerial application of diflubenzuron (25% WP) to treatment plots at a rate of 70.75 g/ha on May 8, 1985 and May 9, 1986 as part of Gypsy moth suppression program in WV. Plots were located in an 8000 ha oak-hickory forest. Untreated plots served as controls.	**Abundance**: No significant differences were observed (p<0.10) in abundance of 21 species of birds examined between treated and control plots. **Diets**: All species in untreated plots ate more Lepidoptera larvae than species on treated plot; difference was significant (p<0.10) in 5 of 7 species. **Foraging**: Vireo foraging areas were 3.1 and 2 times larger on treated areas, compared with untreated areas.	Cooper et al. 1990

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
70 g a.i./ha (1 oz a.i./ac) to cotton, applied in paraffinic crop oil (Dimoil) and water. Sampling took place 1 week after each treatment. Fields 15 ha each.	Assay of populations of predators of bollworms (*Heliothis*): lacewings (*Chrysopa* spp.), ladybird beetle (*Hippodamia convergens*), *Coleomegilla maculata* big-eyed bug (*Geocoris punctipes*), *Nabis* spp., *Orius insidiosus*. Numbers of predators unaffected by 4 treatments 1 week apart. The study did not look at parasite numbers. The authors note that crop oil could have affected some species.	Deakle and Bradley1982
0.3 to 3.3 kg Dimilin 25W/ha [75 g a.i. to 825 g a.i./ha]	No effect on breeding success or growth of nestlings for tree sparrows (*Passer montanus*) or two species of tits (*Parus major* and *Parus caeruleus*). Endpoints examined included number of occupied nest boxes, mean number of offspring, nesting period, mortality of nestlings, and breeding success.	De Reed 1982
110 to 400 g a.i./ha	Honey bee, *Apis mellifera*. No effect from spray on trees on adults or larvae.	Emmett and Archer 1980
38 and 83 g a.i./ha applied in diesel oil (0.54 and 1.19 oz a.i./ac)	Nearly 90% reduction in grasshoppers (nymphs and adults) 7 d. after treatment at higher rate. Low rate had minimal effects on larval grasshoppers. At least one taxon of beetle showed reductions of 50% at highest dose. Possible reduction in trap catches of members of 1 of 3 families (the Gnaphosidae) of ground spiders, at highest dose, 4 weeks after treatment. Reduced populations of Ichneumonids and Braconids in sprayed plots for at least 3 weeks. Possibly due to effects on host species rather than direct toxicity. Tiphiids unaffected by treatments. Predatory wasp reduced in treated plot, possibly a response to prey reduction (grasshoppers).	Everts 1990
Brazil: 250 g a.i./ha (3.6 oz a.i./ac). Applied 3x by mistblower.	No effect on adult levels of predator *Calosoma*, nor on nabids or geocorids.	Heinrichs et al. 1979
70 g a.i./ha (1 oz a.i./ac) in 4.7 l/ha crop oil (Savol) + H2O, applied 6x at 5 d. intervals	Treatments reduced parasitism by *Trichogramma pretiosum* to *Heliothis* spp. by 44% after spray.	House et al. 1980
Apple orchard in Union, CT. 57 g a.i./10 gal water with spreader sticker. Applied with backpack sprayer.	Parasitic wasp *Apanteles melanoscelus* Parasitism rate on treated vs. control trees roughly equal before spray, but lower on treated trees 7 d. after spray (1.81% v. 0.67%). Some adult wasps developed successfully, perhaps those in later stages of development.	Granett and Dunbar 1975

Appendix 3a - 3

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Apple orchard in Brooklyn, CT. 3.5 g a.i./10 gal. water with spreader sticker. Applied w/ backpack sprayer.	Parasitic wasp *Apanteles melanoscelus* 1st application of spray decreased parasitism rate. 2nd and 3rd applications did not.	Granett and others 1976
About 11 and 22 g a.i./ha (0.75% and 1.0% a.i./kg. At 1.1 and 2.2 kg/ha.) Treated bran bait.	30+ spp. of grasshoppers, counted on treated and control fields. Total populations were reduced 28 days after treatment by 60 and 70% at highest rates of application (0.75 and 1.0% a.i./kg; 2.2 kg/ha). Populations reduced <20% at half that rate. Greater effects early instars.	Jech et al. 1993
Cotton fields treated with nine applications of 2 oz a.i./acre (140 g/ha) diflubenzuron (NOS) from June 17-Aug 12	Monitoring of arthropod predator populations: *Geocoris punctipes*, *Nabis* spp., *Hippodamia convergens*, *Coleomegilla maculata*, *Orius insidiosus*, *Chrysopa* spp. Diflubenzuron treatment did not skew the relative abundance of the predators sampled. For 6 days after collection, egg hatch in the laboratory held *H. convergens* was significantly lower in females collected from treated cotton fields, compared with those form untreated cotton fields.	Keever et al. 1977
Backpack application of 8 oz Dimilin 25W or 0.5 pints Dimilin 2L (0.125 lbs a.i./acre in each case) to maturing cotton foliage in Fresno, CA or East Bernard TX	Over 5 weeks, dislodgeable foliar residue ranged from 0.40 μg/cm^2 down to 0.01 μg/cm^2 (limit of quantitation). Regression analysis predicted mean dislodge able residues on cotton leaves of 0.189 μg/cm^2 at 4 hours and 0.180 μg/cm^2 at 24 hours at both locations.	Korpalski 1996a MRID 44081401
Three applications of Micromite 25W via calibrated airblast sprayer to orange trees at a rate of 1.25 lbs (0.3125 lbs a.i./acre) in LaBelle, FL. [0.35 g/ha × 3]	Over 5 weeks, dislodgeable foliar residue ranged from approximately 0.8 to 1.0 μg/cm^2 shortly after the last application and down to 0.22 to 0.48 μg/cm^2 at 35 days post application. Regression analysis predicted mean dislodgeable residues on orange tree leaves of approximately 0.59-0.82 μg/cm^2 at 4 hours and approximately 0l58-0.81 μg/cm^2 at 24 hours at both locations.	Korpalski 1996b MRID 440814012
Diflubenzuron at 150, 450, or 750 g a.i/ha.	Gram pod borer, *Helicoverpa armigera* (Lepidotera: Noctuidae) [crop pest] field collected eggs on gram plants in sprayed and unsprayed plots. % egg mortality: Controls = 13.0% ; 150 g/ha. =39.0% ; 450 g/ha. = 61.0% ; 750 g/ha. = 100.0 %	Kumar et al. 1994

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Site 1: 140 g a.i./ha (2 oz a.i./ac) Site 2: 280 g a.i./ha (4 oz a.i./ac) both in Kamloops, British Columbia	Mites counted in the top 6 cm of soil. About half of the taxa showed significant decreases in abundance from diflubenzuron applications. Overall population unaffected by spraying; increases in some species compensated for decreases in others. Mites in upper 3 cm of soil more severely affected than mites below. Some predators decreased and some increased (trophic level not predictive of susceptibility). 4 species apparently eliminated from site 2, after a year; other species persisted at low levels a year after spray.	Marshall1979
34 and 68 g a.i./ha (0.5 and 0.97 oz a.i./ac)	Honey bee, *Apis mellifera*. Hives placed in gypsy moth treatment blocks. No effects from applications on numbers of adults, larvae, or honey production.	Matthenius1975
Aerial application of Dimilin 25-W at a rate of 70.75 g/ha (2 oz/acre) to 770x770 m (60 ha) plots on May 8, 1985. Plots were separated by at least 150 m to minimize the effects of spray drift. The study area (Morgan Co, WV) was characterized by mature oak-pine and oak-hickory forests. Gypsy moths were mostly 1st and 2nd instars and foliage was not fully expanded at the time of treatment.	Foliage residues: 1 day after treatment = 0.45±0.25 ppm 3 days after treatment =0.31±0.16 ppm 10 days after treatment =0.10±0.06 ppm 21 days after treatment =0.18±0.16 ppm	Martinat et al. 1987
Aerial application of Dimilin 25-W at a rate of 70.75 g/ha (2 oz/acre) to 770x770 m (60 ha) plots on May 8, 1985 and May 9, 1986 Plots were separated by at least 150 m to minimize the effects of spray drift. The study area (Morgan Co, WV) was characterized by mature oak-pine and oak-hickory forests.	Significant, treatment-related reductions were observed primarily in canopy macrolepidoptera and non-lepidopteran mandibulate herbivores. Sucking herbivorous insects, microlepidoptera, and predaceous arthropods were not affected.	Martinat et al. 1988
70 g a.i./ha (1 oz a.i./ac)applied to oak-pine and oak-hickory hardwood.	120 species of spiders (Araneae) and orthopteroid (Orthoptera and Dictyoptera). Significant effects from treatments noted on spider on 1 of 10 sampling dates, and on orthopteroid abundance on 2 of 10 sampling dates. Trend in expected direction on other dates. No change in diversity of these groups. Effect on spiders could be from loss of prey or direct toxicity. Orthropoids picking up from litter that they ingest.	Martinat et al. 1993

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Application of 280 g/ha a.i. Dimilin WP-25 via backpack sprayer to rice field 5 days after emergence of rice leaves out of the water.	Half-life (calculated from first order kinetics) = 27 hours; residues were below detection limit after 96 hours.	Mabury and Crosby 1996

Additional notes on Maybury and Crosby 1996: Sensitized photolysis was the primary route of degradation, although partitioning to sediment and volatilization may have played minor roles in the fate of the compound. Rapid photolysis of DFB to CPU and DFBA. This mixture was as toxic to daphnids as DFB. Photolysis in distilled water is slow. Halftime of alkaline (pH 8.8) photodegradation was 157 hours (2.4 days) and filtered field water (pH 7.4) was 32 hours (1.3 days). Slower rates of photodegradation for CPU and DFBA. Field dissipation halftime for DBF of 27.3 hours (1.1 days) with typical initial increase in concentrations of CPU.

Application	Observations	Reference
30 g a.i./ha, in 4.78 l water (0.43 oz a.i./ac)	Wasp parasite on gypsy moth larvae (*Apanteles melanoscelus*)Parasitic fly in family Tachinidae. Wasp mortality 80% in 2 weeks from field spray. Development halted in most cases, failed to spin cocoons upon emergence, etc. 100% mortality in tachinid parasite. Gypsy moths in 2nd, 3rd, and 4th instar.	Madrid and Stewart1981
Aerial (fixed-wing aircraft) application of Dimilin WP-25 at a rate of 75 g a.i. in 50 L water/ha in A total of 1160 ha of insect-infested forest in Finland in August 1984 in an effort to control the pine looper, *Bupalus piniarius.*A solution of hydroxyethyl cellulose and 15 g sodium bicarbonate in 50 L water/ha was added to formulation to minimize drift, especially near the borders of the treated area.	Residues in run-off water decreased from 5 μg/L one day after spraying to 0.1 μg/L after 2 months. The concentration in water in open pits was 0.1 μg/L 1 and 7 days after application and 0.2 μg/L 1 month after application. After 2 months no residues were detected. All water samples taken from outside the treated area contained < 0.1 μg/L (the limit of sensitivity). No DFB was detected in the treated area the year following application or outside the treated area. Neither 4-chloroaniline nor 4-chlorophenylurea was detected in the water at any time. Residue data for the litter layer, humus layer, pine needles, wild mushrooms, boletus samples, and bilberries are provided.	Mutanen et al. 1988
DFB (25% WP) via handgun to four-tree Valencia orange blocks at a rate of 10 oz a.i./acre. Trees were sprayed to runoff to control citrus rust mite.	Half-lives of DFB surface residues (Exp 1 cool-dry period: March to April): leaves =*essentially none* fruit =118±100 days; soil (middle) = 19±11 days; soil (dripline) = 21±10 days; Half-lives of DFB surface residues (Exp 1 hot-wet period: March to April): leaves + 27±8 days; fruit 18±2days; soil (middle) = levels too low to be detected; soil (dripline) = levels too low to be detected	Nigg et al. 1986

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Aerial application of 70 g a.i./ha Dimilin to experimental watershed (two treated; two controls) in the Fernow Experimental Forest, WV. Soil and leaf litter arthropods were monitored before and after application for a total of 36 months.	Throughout the study, mites (49%) and springrtails (28%) dominated the soil core sample. A total of 19 taxonomic groups were suitable for statistical analysis. No significant treatment effects were observed, based on total organism counts or counts by trophic categories ($p<0.05$).	Perry et al. 1997 Perry 1995b

Additional Notes on Perry et al. 1997: No significant treatment-related effects for populations of major taxonomic groups, except for Araneae (spiders) were observed. Analysis of leaf-litter bags also indicated no significantly differences in total numbers of invertebrates or in trophic categories between treated and untreated watersheds during the 12-month post treatment study. There appeared to be an indirect effect on spiders as a taxon, which may have resulted from changes in prey populations.

Application	Observations	Reference
Aerial application of Dimilin 25W at a rate of 33.23 g a.i./ha in 9.4 L/ha to a 20-ha forest block in central PA. Leaf samples were collected from the upper and lower canopies of 27 oaks and understory within the block on the day of application, May 29, 1991. Canopy leaves were also collected on May 31, June 10, July 29, and September 26, 1991.	On the day of application, DFB residues on the upper canopy, lower canopy, and understory averaged 81.18, 39.65, and 8.35 ng/cm^2. DFB residues on canopy leaf residues were: 14.83 ng/cm^2 (day 2 post spray) 16.75 ng/cm^2 (day12 post spray) 12.84 ng/cm^2 (day 61 post spray) 11.20 ng/cm^2 (day 120 post spray) DFB residues on litter-leaf sample collected after leaf senescence 169 and 323 days after treatment contained measurable amounts of DFB in 51 and 59% of the samples, respectively.	Prendergast et al. 1995
Three cover sprays of diflubenzuron (NOS) at 3.7 or 7.4 g a.i./100 L in a pear orchard in northern CA. [Data to calculate application rate in g/ha not given]	DFB treatment had no direct effect on pear psylla (pest species), did not induce phytophagous mites, and was weak, compared with the synthetic pyrethroid, fenvalerate against the codling moth.	Riedl and Hoying 1980
0.5 and 2 oz a.i./ac, w/ crop oil, sprayed 8 times on cotton. [35 to 140 g/ha × 8]	Direct spray of bee hives. No effects noted on adult mortality, rate of larval growth, brood production, or honey or wax production. No residues in wax or honey. Not caged study, so bees could have foraged outside of spray area.	Robinson 1978,1979
Aerial application of oil formulation of DFB (Dimilin 45 ODC) on August 31st in a conifer forest in the north of Spain at a dose of 56.3 g a.i./ha a(125 cm^3 Dimilin in 5 L diesel oil) (volume rate of application = 5 L/ha). The day of application was clear with no rainfall in the previous 48 hours.	DFB persisted for 10-12 weeks on the foliage of the conifer forest; 55-80% of the insecticide was removed from the foliage within 22-30 days after treatment; aerial application resulted in residue levels of 867.5-1824.4 ng/g, depending on the forest characteristics. 2,6-difluorobenzmide was the only metabolite detected and persisted only until the first rainfall.	Rodriguez et al. 2001

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Aerial application of Dimilin 25-W at a rate of 70.75 g/ha (2 oz/acre) to 770x770 m (59.2 ha) plots on May 9, 1986 Plots were separated by at least 150 m to minimize the effects of spray drift. The study area (Morgan Co, WV) was characterized by mature oak-pine and oak-hickory forests.	Diets of five species of forest birds were significantly different between treated and untreated plots. Treatment generally decreased the biomass of Lepidoptera larvae and increased the biomass of other orders (Homoptera, Diptera, Coleoptera, etc.).Two species of birds in treated sites had decreased total gut biomass. The investigators conclude that DFB has an indirect adverse effect on forest birds by reducing the availability of Lepidoptera larvae.	Sample et al. 1993a
Aerial application of Dimilin 25-W at a rate of 70.75 g/ha as part of a gypsy moth suppression program in WV.	Treatment adversely affected Lepidoptera resulting in decreased abundance and species richness; no effects were observed among Coleoptera, Diptera, or Hymenoptera. Trap catches of 3 families of Hymenoptera were unaffected, including two parasitic families, Ichneumonidae and Braconidae.	Sample et al. 1993b
Application of Dimilin *on a regular basis* (i.e., 8 applications between May 16th and December 14th 1977) to a small citrus grove in which there were two bee hives.	The hives remained in the same location throughout the study and were covered with plastic as a means of protection. There were no adverse effects on brood development of honey bees.	Schroeder 1978a MRID 00099731

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Aerial application of 350 g a.i./ha diflubenzuron to a commercial citrus grove to control *Diaprepes abbreviatus*	Residues ***in ppm*** on fruit harvested 27 days after the 6th application were: 0.34 on unwashed fruit; 0.11 on washed fruit; 0.26 on dried pulp; 0.31 on peel fruit; 0.12 on chopped peel; and 20.55 in oil.	Schroeder 1980

Additional Notes on Schroeder 1980: No detectable residue (<0.05) of DFB was found in the finisher pulp, fruit juice, pressed liquor, molasses, prewash or afterwash water, and emulsion water fractions. No detectable residue (<0.05) of 4-chlorophenylurea or 4-chloroaniline was found in the citrus fractions or in the prewash or afterwash water. The total sealed brood in honey bee (*Apis mellifera*) was not significantly different from control at 7 months and there was no detectable residue (<0.05 ppm) of DFB,CPU, or 4-chloroaniline was found in the honey obtained after 8 aerial sprays. Populations of non-target citrus pests and beneficial species were not affected by the spray program.

Application	Observations	Reference
Sour orange (*Citrus aurantium*) trees sprayed to runoff with Micromite 25W at 149 or 298 g a.i./1000 liters Efficacy study.	Diflubenzuron, formulated as Micromite 25W, significantly affected the reproductive potential of the sugarcane rootstock borer weevil, *Diaprepes abbreviatus* (pest of sugarcane and citrus).	Schroeder 1996
Aerial application of Dimilin formulated as 25% wettable powder at the rate of 140g/ha to 770 m square plots with a buffer strip of at least 150 m between adjacent plots in May 1985 and 1986.	Estimates of density of white-footed mouse, *Peromyscus leucopus*) did not differ significantly (p>0.05) between treated and untreated areas. Juvenile/adult female ratios on untreated areas were significantly higher (p<0.05), compared with those on treated sites. Mice on treated sites consumed less Lepidotera prey, compare with controls (p<0.05); however, the total amount of food consumed per mouse did not differ significantly between treated and untreated areas (p>0.05). There were no treatment-related adverse effects on body measurements, weight, or fat content.	Seidel and Whitmore 1995
Aerial application of Dimilin (NOS) at a rate of 140 kg/ha. The application rate is presumably a.i. but this is not specified in the publication.	No effect on bird populations that could be attributed to diflubenzuron. Various changes in the populations of different bird species are discussed but detailed data are not reported in the publication.	Stribling and Smith 1987
Simulated aerial application of diflubenzuron in acetone or in fuel oil each at 90 g a.i. in 18 L/ha to spruce foliage (*Picea glauca*).	The residue levels 1 hour after application varied, respectively, from 23.8 to 30.6 µg/g in foliage and from 3.08 to 4.60 µg/g in litter. Forty-five days after spraying the residue levels in foliage were 0.80 and 3.9 µg/g, respectively, for acetone and fuel-oil formulations.	Sundaram 1986 MRID 00161955 Sundaram 1986

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Simulated aerial spray application of technical grade DFB in acetone formulation with tracer dye or in fuel oil with tracer dye at a rate of 90 g a.i. in 18 L/ha to white spruce foliage of uneven height. The forest floor was flat and covered with grass and moss patches.	The half-lives for DFB in foliage, litter, and soil for the acetone-based formulation were 9.30, 8.36, and 7.49, respectively. 45 days after application, the residue levels in foliage were 0.80 µg/g (fresh weight) for the acetone-based formulation. There were no detectable residues in litter or soil on the 45th day post application of the acetone-based formulations.	Sundaram 1996
Soybeans in S.C. treated with 281 or 562 g a.i./ha (4 or 8 oz a.i./ac)	Significantly fewer nabids and geocorids on treated v. control sites.	Turnipseed et al. 1974
Aerial application of 8 oz Dimilin WP-25/acre (equivalent to 0.0625 lb/acre) to 10-acre mixed hardwood-conifer forested plot near Boone N. Carolina, which consisted of a stream, two stream pools, and a stream-fed pond outside the treated area. Sandy loam soil. Cumulative rainfall of 43.1 cm (16.9 inches) over a 1 year period. Daily rainfall and temperature data are given.	Initial concentration on leaves in canopy of 13 ppm on hardwood and 5.9 ppm on conifer. Initial concentrations on understory vegetation of about 0.13 ppm that increased initially as with litter. Diflubenzuron was rather persistent on leaf litter. Initial residues of 0.07 ppm. This increased over a 60 day period, probably due to drying of litter, washoff of DFB, and leaf fall from canopy.	Van Den Berg 1986 MRID 00163853

Additional Notes on Van Den Berg 1986: A single application resulted in initial water concentrations in treatment area of 0.127-0.203 ppb. Declined to 0.029-0.045 ppb after one day. No detectable contamination in an adjacent pond after heavy rains. Initial soil concentrations of 0.02 ppm and 0.03 ppm after a 6.5 cm rain (probably washoff). No DFB in 3"-6" soil samples. The study authors conclude that the effects on the mites and collembolans present at the time of application were insubstantial. In general, fewer of each group on treated than untreated sites. The data are somewhat difficult to interpret because of erratic capture patterns over time the populations of collembolans were different at the control and treated sites prior to treatment. [NOTE: Data on other species presented in Tables 10 and 11 but the numbers of insects are too small for analysis. Species list in Table 11 cut off on fiche]

28 g a.i./ha (0.4 oz a.i./ac)	Wasp parasite on Gypsy moth larvae (*Cotesia melanoscelus*) Pathogen: gypsy moth nuclear polyhedrosis virus (NPV). Numbers of the wasp no different on Control v. treated plots. Incidence of NPV significantly lower in treated plots. Late instar spraying may preserve larvae long enough for parasitoid to complete development. Earlier spraying kills host too quickly, hence parasitoid as well. NPV lower in treated plots because fewer Gypsy moths to transmit virus.	Webb et al. 1989

Appendix 3a: Summary of terrestrial field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
application of 2 or 4 oz a.i./acre [140 to 280 g/ha] to plots with large, active nests of yellowjackets	Yellowjackets (*Vespula* or *Dolichovespula*). Treatment decreased populations and the effect was readily observed during the following year. No effects observed on Mound-building ants (*Formica)*	Weiland 2000 MRID 45245403
560, 280, and 140 g a.i./ha (8, 4, and 2 oz a.i./ac). 2 and 3 treatments. Handgun and air-carrier sprayer.	Nearly twice as many *Psylla* predators and parasites per season in the lowest application rate. Higher rates resulted in higher populations of the pear psylla.	Westigard 1979
Aerial application of 0.03lbs a.i./acre Dimilin 25WP to Appalachian forest ecosystem during 1991 season (20 trees representing 7 species) in WV Univ. Experimental Forest.	Residue on leaves: significant loss of DFB from foliage ranging from 20 to 80% within the first 8 days after application; remaining DFB generally persisted for the rest of the growing season until leaf fall, at which time 13/20 treated trees retained more than 20% of the original pesticide applied.	Wimmer et al. 1993
Dimilin (TH-6040) formulated as dispersable powder (a.i. 25% by weight) applied aerially at the rate of 0.28 kg a.i./ha (0.25 lbs a.i./acre) to a Douglas-fir forest ecosystem in British Columbia	Treatment decreased the total number of flying insects and the effect was sustained throughout the study period, with the greatest impact observed on midges and gall gnats. Mosquitoes were completely wiped out as a result of treatment.	Wilson and Wan 1977a MRID 00095419 Wilson and Wan 1977b MRID 00129973

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Two applications (NOS) of granular diflubenzuron at 0.11 kg a.i./ha (about 3.7 µg/L) or 0.22 kg a.i./ha (about 7.4 µg/L) to residential-recreational lakes in San Bernadino County (June 1967 - January 1977)	**At 0.11 kg a.i./ha application**: *Daphnia pulex* and *Daphnia galeata*: 62-75% decrease in population during 7 days after treatment; populations recovered in the second week after treatment.	Ali and Mulla 1978a

Additional Notes on Ali and Mulla 1978a: 0.11 kg/ha continued. *Diaptomus* spp. (copepods): 30% decrease in population observed 2 days after treatment. *Hyalella azteca* (amphipods): 97% decrease in population observed 3 weeks after treatment; populations remained below pretreatment levels throughout 8-9 week evaluation. Treatment had no detectable effects on *Cyprihnotus* sp.(seed shrimp), *Cyclops*, or *Bosmina longirostris* (Cladocera).

At 0.22 kg a.i./ha application: *Daphnia pulex* and *Daphnia galeata*: completely eliminated for 3 weeks after treatment. *Diaptomus* spp (copepods): populations decreased to 0 within 7 days after treatment, but recovered completely *soon thereafter*. *Hyalella azteca* (amphipods): 30-100% decrease in population during 2 ½ months after treatment. *Cyprihnotus* sp.(seed shrimp): population *stressed for only 2 weeks*. *Oligochaete* (mostly *Naididae* found in marine, brackish, and freshwater habitats): no significant effects observed at either treatment level.

Application	Observations	Reference
Two spray application of diflubenzuron (25% WP) to entire surface of residential-recreational lake in Riverside County at a rate of 156 g a.i./ha-surface (about 0.012 ppm) in April and August 1977.	**First application (April)** *Daphnia leavis* and *Ceriodaphnia* sp: population eliminated within 1 week with **no recovery** 6 months after treatment.	Ali and Mulla 1978b

Additional Notes on Ali and Mulla 1978b: *Bosmina longirostris* (cladocerans): population eliminated within 1 week with recovery after 11 weeks. *Cyclops* sp. (crustaceans): population eliminated within 1 week with recovery within 6-7 weeks. *Diaptomus* spp. (copepods): population eliminated within 1 weeks with recovery after 4 months. *Hyalella azteca* (amphipods): population eliminated within 4 weeks with **no recovery** 6 months after treatment. *Caenis* sp. [Hemeroptera (mayflies, immature)]: elimination within 3 weeks with recovery within 6-7 weeks. *Physa* sp. (sinistral snails, referred to as pond snails or pouch snails): no adverse effects. *Cypridopsis* sp.(bivalve): no adverse effects. **Second application (August)** *Bosmina longirostris* (cladocerans): population eliminated after 1 week; reappearance in small numbers 8-9 weeks after treatment. *Cyclops* sp. (crustaceans): population eliminated within 1-2 weeks with recovery after 4 weeks. *Diaptomus* spp. (copepods): population absent prior to treatment; reappearance in small numbers 1-2 months later. *Caenis* sp. [Hemeroptera (mayflies, immature)]: elimination within 2-3 weeks with recovery within 4-5 weeks.
Study does not provide monitoring data. See Ali et al. 1988 below.

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Application via airblast sprayer of Dimilin 25 WP at a rate of 0.56 kg a.i./ha to 0.8 ha of citrus immediately surrounding a pond located in Winter Garden, FL. The pond was exposed to air-drift diflubenzuron from surrounding citrus area commercially treated for the control of citrus rust mite. The control pond was located 0.4 km NE of the exposed pond.	No apparent adverse effects on zooplankton and benthic invertebrates in treated pond. Minor reductions of copepods and cladocerans during post-treatment period most likely due to short life cycle, seasonal population changes, and possible sampling deficiencies. Largest detected diflubenzuron residue = 197 ppt, 2 days after application with levels returning to trace amounts (<27 ppt) by day 14 after application. Specifics on the pond: circular,2 ha at the surface; 3/4 of its border was lined by citrus trees.	Ali et al. 1988
One surface application (via rowboat hand sprayer)of Dimilin (25% WP in 20.5 L water) to each of three ponds (0.6-0.2 hectares) at rates of 2.5, 5, or 10 ppb a.i. in California to control gnats (*Chaoborus astictopus*)and one application to a large lake at a rate of 5 ppb a.i. Surface area of ponds ranged from 0.06-0.2 ha; ponds were rectangular in shape with steep sides and flat bottoms.	Treatment was effective against gnats, decreasing larval abundance by 99%. Crustacean zooplankton populations declined precipitously at all application rates, but the effects were not permanent. Cladocerans were more susceptible than copepods and required longer recovery period. *Anabaena* sp (blue-green algae) decreased by approximately 70% within 2 weeks after treatment and remained at low levels throughout the study period; treatment seemed to have no effect on diatoms or green algae. The bioaccumulation of diflubenzuron in bluegill sunfish diminished rapidly as the residues in water decreased. No effect on growth of bluegills.	Apperson et al. 1977 MRID 00099897 Apperson et al. 1978

Additional Notes on Apperson et al. 1977, 1978: The investigators indicate that no severe or permanent nontarget effects were observed in this study. ***Residues***: In pond water, residues in the 10 and 5 ppb ponds 1 hour after treatment ranged from non-detectable to 23.6 and 32.2 ppb and averaged 9.8 and 4.6 ppb, respectively and residues levels in the 2.5 ppb pond at 4 hours after treatment ranged from N.D. to 8.3 ppm with an average of 1.9 ppb. Maximum values in bottom water samples in the 5 and 2.5 ppb ponds occurred at 4 hours and 14 days and averaged 5.3 and .5 ppb, respectively. The DFB residues declined steadily soon after treatment and at the end of the study, levels averaged 0.2, 0.3, and 0.5 ppb for the 10, 5, and 2.5 ppb ponds, respectively. No residues were found in the sediment samples.

Applications to test ponds at 1X and 4X of the typical application rate.	No effects on invertebrates or fish. [This study is poorly documented and should be given minimal weight.]	Birdsong 1965
Four applications of Dimilin W25 to ponds located in Salt Lake County Utah between 7/14/15 and 10/7/75	Algae (*Plectonema*) degraded 80% of the TH-6040 in a 1-hour incubation period. Degradation products were primarily p-chlorophenyl urea and p-chloroaniline.	Booth and Ferrell 1977 MRID 00099884

Additional Notes on Booth and Ferrell 1977: Bacteria (*Pseudomonas* sp.) accumulated "rather large amounts" of TH-6040 from the incubation media when used as the sole carbon source. No degradation products were observed in the media. Channel catfish id not bioaccumulate DFB residues from treated soil in a simulated lake ecosystem constructed in the laboratory.

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Repeated, pulsed exposures of diflubenzuron on twelve outdoor aquatic mesocosms (0.1 ha each). Random assignment of mesocosms (four/treatment) to either monthly (five total 10 µg/L applications) or biweekly (nine total 10 µg/L applications). Direct and indirect impacts on mesocosms were measured over 16 weeks after treatment.	Within 4 weeks after monthly and biweekly treatment, direct effects on Cladocerans, Copepods and Rotifers included 5-fold decrease in total numbers, 2-fold decrease in species richness, and 2-fold increase in zooplankton. Direct reductions in the numbers of invertebrate grazers caused indirect increases in algal biomass. Decreased invertebrate numbers resulted in decreases in invertebrate food resources that resulted in a 50% reduction in both biomass and individual weights of juvenile bluegills. There were no statistically significant impacts observed on adult bluegills or largemouth bass for the duration of the study.	Boyle et al. 1996

Additional Notes on Boyle et al. 1996: DFB concentrations averaged 9.9 µg/L 24 hours after chemical application. The half-life of disappearance of DFB from water, calculated across all ponds and dates using a negative exponential decay model was 2.33 days (range = 1.76-2.96 days). There were no significant differences in DFB dissipation rate between treatment type (monthly or weekly; $p \geq 0.5815$) or season (early or late in the study; $p \geq 0.4728$).

Application	Observations	Reference
aerial application of 35 g/ha in Canada	No toxic effect on bullheads or sunfish.	Buckner et al. 1975
Two ground spray applications (at 2-week intervals) to each of two CA sites (one in Tiburon, Marin County and one in Roseville, Placer County). The first Tiburon application = 13 g/ha (0.19 oz/acre) and the second Tiburon application = 35g/ha (0.5 oz/acre);both Roseville applications = 26.25 g/ha (0.38 oz/acre) of Dimilin 25W (diflubenzuron 25% a.i.). Foliage was sprayed to the point of drip. Each site was approximately 0.8 ha. The applications were made in March-April 1990.	*Foliage*: DFB concentrations from 0 (not detected) to 18.31 µg/g immediately after the second application; and from 0(not detected) for background to 0.252 µg/cm^2 leaf area immediately after the second application. 28 days after the second application, the DFB concentration decreased sharply suggesting possible degradation during that period, but no samples were collected during the 28 days to document a degradation trend. *Air*: During 3 of the 4 applications, DFB concentrations in air ranged from 0.0106 to 0.0187 µg/m^3. DFB was not detected in any background air samples or in any 1 day post application air samples (i.e., DFB was detected in air only during application periods). *Water*: Samples collected from streams and water bodies in and near the treated areas on the day prior to application, immediately after each application, and 7 days after each application showed no detectable levels of DFB (minimum detection limit = 0.5 ppb).	Carr et al. 1991

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Application (NOS) of diflubenzuron to five experimental, rectangular ponds in Lakeport, CA, yielding a mean concentration of 13 µg/L DFB. Each pond had a surface area of about 0.01 ha (1 ha =10,000 m^3) and a depth of 1.2 m.	**Residues in water** decreased below detectable limits (0.2 µg/L) by 14 days after treatment; at one hour after treatment, the mean concentration of DFB in water was 13.2 µg/L.	Colwell and Schaefer 1980

Additional Notes on Colwell and Schaefer 1980: Cladocerans: most abundant species included *Ceriodaphnia*, *Diaphanosoma*, *Chydorus*, *Bosmina*, and *Daphnia*, all of which showed population reductions in all treated ponds within a few days of DFB application. **Copepods**: abundance of naupli decreased in all ponds after treatment and returned to pretreatment levels from 7 days to >4 weeks after treatment. *Diaptomus* (filter feeders) and *Cyclops* were similar in their susceptibilities to DFB, although in most of the treated ponds, *Diaptomus* populations recovered more rapidly than *Cyclops* populations. **Rotifers**: *Brachionus*, *Keratella*, and *Hexartha* populations increased in treated and control ponds during the first 8 days after treatment. *Asplanchna*, which are mostly predatory increased from 0.18 to 0.43 organisms/L after treatment. **Fish**: Young-of-the year black crappie, *Pomoxis nigromaculatus*, and brown bullhead, *Ictalurus nebulosus*, accumulated DFB and then eliminated all residues by day 7 after treatment. No fish mortalities occurred after treatment. For 1 month after treatment, the stomach content analyses of exposed fish indicated major alterations in diet. Neither growth rates or general condition of the fish 3 months after treatment differed from those of controls.

Application	Observations	Reference
Six aerial applications of 28 g/ha of diflubenzuron over 18 months (June 1974 through Sept 1975) to a Louisiana intermediate marsh	Treatment resulted in statistically significant differences in population density of non-target aquatic organisms (target organism - mosquito), compared with controls, but none of the affected organisms were completely eliminated from the ecosystem. The investigators speculate that the untreated marsh areas would provide populations of aquatic organisms that could repopulate the treated areas.	Farlow 1976 MRID 00099678 [Also published as Farlow et al. 1978]
Six applications of diflubenzuron (28 g a.i./ha) in a Louisiana coastal marsh over an 18-month period.	Statistically significant differences in the population density of aquatic organisms; however, none of the organisms affected were completely eliminated from the ecosystem.	Farlow et al. 1978

Additional Notes on Farlow et al. 1978: Significant populations decreases observed in five taxa: nymphs of *Trichocorixa louisianae* (water boatman) and *Buenoa* spp.(backswimmers), Coenagrionidae naiad spp.(damselflies), *Berosus infuscatus* adults (water beetles), and *Hyalella azteca* (amphipods). Significant increases were observed in populations of 15 taxa exposed to diflubenzuron, i.e., *Physa* sp. (snails), *Ceanis* sp. and *Callibaetis* sp. naiads (mayflies), *Noteridae* larvae (water beetles), *Hydrovatus cuspidatus*, adults (water beetles), *Hydrovatus* sp. larvae (water beetles), *Dytiscidae* larvae (great diving beetle), *Mesovelia mulsanti* adults (water treaders), *Trichocorixa louisiana* adults (water boatman), larvae of Chironomidae (non-biting or true midges), Ephydridae (shore flies), Dolichopodidae (long-legged flies) and Tabanidae (horseflies), as well as mosquito fish (*Gambusia affinis*) and American flag fish (*Jordanella floridae*). The 27 remaining aquatic organisms (members of the Hemiptera, Coleoptera, Mysidacea, Decapoda, Diptera and Odonata) showed no statistically significant differences, compared with untreated populations.

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Aerial application of Dimilin 4 L at a rate of 35.1 g a.i./ha to two stream catchments in the Fernow Experimental Forest, WV in May 1992.	Treatment decreased the adult emergence of stoneflies, *Peltroperla arcuata*, during the first 4 months after treatment, compared with untreated catchments. Adults populations of other species did not decrease in the treatment catchments during the period of study.	Griffith et al. 1996

Additional Notes on Griffith et al. 1996: The investigators speculate that additional detritivourous species might have shown an adverse effect if the monitoring were extended through the period after treated leaves entered the streams. Stoneflies *are considered to be obligate large-particulate organic matter feeders and like ingested diflubenzuron from leaves that fell earlier in the year, thus ingesting diflubenzuron.* Diflubenzuron was not detected in water samples taken from the streams following treatment, perhaps due rainfall just prior to treatment.

Application	Observations	Reference
Aerial application of Dimilin 4 L at a rate of 35.1 g a.i./ha to two stream catchments in the Fernow Experimental Forest, WV in May 1992. During 1993, no additional diflubenzuron was applied to any of the watersheds.	The investigators tested the hypothesis that diflubenzuron affected adult flight following emergence during the year following abscission and possible ingestion of the treated leaves. The flight of the stonefly, *Leuctra ferruginea*, was reduced in the treatment watersheds, compared with the reference watersheds during the year following abscission of the treated leaves. Adult flight of other species did not decrease in the treatment watersheds during 1993.	Griffith et al. 2000
Aerial application of Dimlin 4L at a rate of 70 g a.i./ha to two of four watersheds in the Fernow Experimental Forest, WV.	Stream macroinvertebrate taxa that had reduced mean densities in treated watersheds ($\propto$ = 0.05) included the stoneflies, *Leuctra* sp. and *Isoperla* sp., mayflies, *Paraleptophlebiaspia* sp., and cran flies, *Hexatoma* sp. Shredders, the dominant functional feeding group also had reduced mean densities in treated watersheds. Densities of Oligochaeta (aquatic worms) and Turbellaria (flat worms) increased in treated watersheds.	Hurd et al. 1996
Spray application (via portable garden sprayer) of Dimilin (25% wettable powder) at recommended rate of 0.03 lbs a.i./acre or 4X application rate to each of two 10-acre earthen ponds (avg depth of 3 ft). 4X applications were made biweekly beginning in early Feb.	No appreciable mortality of fish or clams in any of the ponds. Treatment significantly decreased *Daphnia spp*. populations and virtually eliminated dipterans. Olgochaete populations, which increased in the control pond during the study, decreased in response to treatment.	Jackson 1976 MRID 00099891

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Aerial application of 0.06 lbs a.i./acre (67.26 g/ha) Dimilin to 75-acre watershed containing small, first order stream.	Dimilin reached the stream channel during aerial application and as a result of wash-off from the foliage during several subsequent rainfall events. DFB levels (measured) exceed the acute (1.0-1.8 ppb) and chronic (60 ppt) toxicity doses for tolerant taxa, like Ephemeroptera (mayflies) and Plecoptera (stone flies). The residence time for Dimilin in these high-gradient streams was very short, and as a result of the short residence time or low concentrations, toxic effects were not evident.	Jones and Kochenderfer 1987
Spray application of 60 g a.i./ha diflubenzuron to five Sahelian temporary ponds (surface areas 0.36-0.65 ha) conducted in mid-September (half-way through rainy season) in vast savannah-type cultivated region in Senegal's ground-nut producing area. Table 1 provides a summary of wind speed, surface area treated, quantity of formulation applied in mL and calculated application rates at each of the 5 treated ponds.	Average initial concentrations in water = 10.4 µg/L, with an estimated half-life of <24 hours. DFB only affected crustaceans (i.e., cladocerans and fairy shrimp) in the treated ponds. DFB virtually eradicated the abundant fairy shrimps, *Streptocephalus* spp., and the populations did not recover despite the rapid disappearance of DFB. In general, cladocerans populations were initially wiped out (densities dropped to 0) after DFB treatment but returned to normal values in 3-4 weeks (*M micrura*), 4-6 weeks (*D senegal*), or 6-7 weeks *C quadrangula*).	Lahr et al. 2000
Application (via backpack sprayer) of Dimilin WP-25 at 280 g/ha a.i. 5 days after emergence of rice leaves out of the water to sic 20 m^2 flooded plots in June 1991 and 1992.	Field dissipation rates were similar for the six replicate plates with a half-life (1st order) of 27 hours; residues dropped to below detection limit after 96 hours. Residues in sediment were 0.16 µg/g (after 24 hours), 0.10 µg/g (after 48 hours) and 0.08 µg/g (after 72 hours); residues were below detection limit after 4 days.	Mabury and Crosby 1996
Spray application (via hand sprayer)of Dimilin 25% WP (TH6040) to semi-natural pools at the Univ. Delaware Experimental Farm to study the cumulative toxicity to killifish (3 applications over 29 days) and crustaceans (one 13-day test and one 15-day test). Applications were made at the rate of 0.01, 0.04, 0.10, and 0.20 lbs a.i./acre – i.e., up to 224 g/ha.	There was no significant mortality in killifish after three successive applications of Dimilin at 0.01-0.20 lbs a.i./acre. Behavioral responses were similar to those of controls. In the first test involving crustaceans, grass shrimp mortality was 83.3% (p<0.01) after the first application of 0.20 lbs a.i./acre. After two applications the average mortality (p<0.01) was 86.6% at 0.4 lbs a.i./acre and 100% at 0.10 and 0.20 lbs a.i./acre.	McAlonan 1975 MRID 00099895

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Additional Notes on McAlonan 1975: In the second test involving crustaceans, grass shrimp average mortality ($p<0.01$) was 91.6% at 0.4 lbs a.i./acre, 96.6% at 0.10 lbs a.i./acre, and 98.3% at 0.20 lbs a.i./acre. In the first test involving crustaceans, fiddler crab average mortality was 60.0% and 46.6% ($p<0.01$) after one application of 0.10 or 0.20 lbs a.i./acre, respectively. After two applications of 0.04 and 0.10 lbs a.i./acre the average mortality ($p<0.01$) was 53.3% and 66.6%, respectively. In the second test involving crustaceans, fiddler crab average mortality ($p<0.05$) was 46.6% at 0.4 lbs a.i./acre, 60.0% at 0.10 lbs a.i./acre, and 66.6% at 0.20 lbs a.i./acre.		
Aerial application of 0.56 kg a.i./ha (8 oz a.i./acre) Dimilin 25 WP to a citrus grove in Florida with an experimental pond	DFB was not observed in water samples at quantitative methods 1 hour post application; maximum levels occurred at 1 and 2 days post application, primarily along the line of drift. Pad data indicate that the pesticide drift deposited along a small portion of the shoreline at a rate 7% of the theoretical application rate ($38 \div 104 \div 5.6$) and the drift continuing out into the pond was as much as 0.8% the application rate ($4.4 \div 104 \div 5.6$).	Nigg and Stamper 1987 MRID 40197002
Dimilin 4L at a rate of 80g/ha (0.03 lb/acre) in two forest watersheds	Decreased populations of stoneflies in treated areas. In untreated areas, the populations of stoneflies increased. After treatment, populations of roundworms, flatworms, and segmented worms were higher in treated areas.	Perry 1995a
Aerial application of 0.0624, 0.125, or 0.25 lbs/acre Dimilin to plots in Oxbow, Maine that included four streams. [up to 280 g/ha]	Effects of a single application (to control spruce budworm) on stream invertebrate fauna (*Trichloptera*, *Plecoptera*, *Ephemeroptera*, *Diptera*, *Odonata*, and *Coleptera*). No pattern of decrease in any individual genus; no treatment-related increase in drift among samples; no treatment related changes in the number of dead drift when collections were made 1-2 days after treatment.	Rabeni and Gibbs 1975 MRID 00159905
Application (NOS) of 1.25 ppm Dimilin 25WP for 1 hour on July 13, 1984 to four points of the Kokawa River in the Izu Peninsula to control blackflies. The gradient of the river was approx. 2% and sampling stations are located between 50 and 250 m above sea level.	Most invertebrates were eliminated within 2 weeks, while Hydropsycidae (caddisfly) died out gradually. Adults of Elmidae (Riffle beetles), previously absent, appeared 1 week after treatment in large numbers at the uppermost of the treated region. No fish mortality was observed.	Satake and Yasuno 1987

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Aerial application of Dimilin WP-25 at a rate of 70 g a.i. in 10, 5, and 2.5/H to three spray blocks in a mixed boreal forest near Kaladar Ontario Canada. Water, sediment and aquatic plants were analyzed for DFB residues. Ponds appear to have been directly sprayed.	The duration of detectable DFB residues in water, sediment, and aquatic plants differed for each substrate but in all cases was less than 2 weeks. There was significant mortality in two groups of caged pond invertebrates (amphipods and corixidae [water boatman]) 1-6 days after treatment. Three taxa of littoral insects (mayflies, dragonflies, and damselflies) were significantly reduced in abundance in treated ponds 21-34 days post treatment but recovered to pre-treatment levels by the end of the season. Cladoceran and copepod populations were reduced 3 days after treatment and remained suppressed for 2-3 months.	Sundaram et al. 1991
5 monthly surface applications of 0.05 lbs a.i./acre Dimilin (25% WP) [56 g/ha] to artificial pond containing mosquito fish (*Gambusia affinis*)	No adverse effects on population growth of fish.	Takahashio and Miura 1975 MRID 00016545
2x application of Dimlin W-25 at a rate of 0.03 lbs a.i./acre at 14-day interval to an outdoor 750 gallon aquarium containing pond water and sediment, bluegill sunfish, clams, and crayfish; fate of diflubenzuron in all elements of the simulated ecosystem was monitored for 42 days from initial treatment.	Rapid dissipation of DFB (half-life < 12 hours); rapid accumulation of compound by fish and clams with rapid elimination (plateau of approx. 55 ppb by day 27 which was maintained for the duration of the experiment); fish samples contained several degradation products (CPU and DFB represent the only organo-extractable residues; clam samples contained only DFB; crayfish did not accumulate any of the compound during the week after the initial treatment.	Thompson-Hayward Chemical Co 1979 *In*: Technology Sciences Group Inc. 1998 MRID 44460702
Aerial application of Dimilin at a rate of 4.5 kg/ha (4 lbs granules/acre) to a tidal flood plain of the Fraser River in British Columbia in June 1976 . The organisms in the tidal flats of the Fraser River at the time of the study included crustaceans (zooplankton), insects, water mites and bugs, snails, and clams.	**Residue**: Dimilin, which was detected in the water up to 71 days after treatment, peaked at 1.8 ppb 8 days after application and decreased slowly to a minimum level of 0.24 ppb at 2 months after application. In mud, Dimilin peaked at 5.66 ppb 4 hours after application and decreased to a minimum level of 1.3 ppb by 2 months after treatment. **Biological effects**: Treatment arrested mosquito development but also decreased the population of zooplankton and suppressed the emergence of non-target insects of the same order as the mosquitoes.	Wan and Wilson 1977 MRID 00095416
Dimilin forestry spray at 67 g DFB/ha	No effect on aged brown trout in stream from day -7 to day +6. Observations along length of stream revealed no indication of fish mortality. Based on population estimates 6 weeks following application, no delayed effects on fish populations.	White 1975

Appendix 3b: Summary of aquatic field or field simulation studies on diflubenzuron and its formulations.

Application	Observations	Reference
Broadcast foliar spray at rate of 0.25 lbs a.i./acre of Dimilin 2L to rice paddy test plots in Arkansas and California 40 days after rice planting.	DFB and its metabolites (DFBA and CPU) dissipated rapidly in the aquatic environment and there was no downward movement of DFB or its degradation products in aquatic soil/sediment.	Willard 1999 MRID 45009601
Broadcast spray application of Dimilin 25W to entire surface area of pond (containing fish) at a rate of 0.36 lbs a.i./acre.	calculated half-life for DFB in water = 5.4 days calculated half-life for DFB in soil/sediment = 8.6 days.	Willard 2000a MRID 45191001
Benthic communities in outdoor experimental streams , concentrations of 1 or 10 mg/L diflubenzuron for 30 minutes	No drift of macrobenthos was induced at the time of application. However, diflubenzuron affected the emergence of all species examined. High larval mortality for a species of chironomid was observed directly in the stream treated with diflubenzuron, where numbers of mayfly nymphs and caddisfly larvae were also decreased	Yasuno and Satake 1990

Appendix 4: Toxicity of diflubenzuron and diflubenzuron formulations to birds

Species	Nature of Exposure	Exposure Time	Effects	Reference
Single Dose				
Mallard ducks, males and females, 10 birds/dose group	single gavage doses ranging from 1000 to 5000 mg/kg bw TH-6040 (99.4% pure)	single dose	No mortality, no signs of abnormal behavior or toxicity, and no gross pathological changes to organs. NOEC = 5000 mg/kg bw	Roberts and Parke 1976 MRID 00073936
Bobwhite quail	5000 mg/kg single gavage dose	single dose	LD_{50} >5000 mg/kg bw	U.S. EPA/OPP 1997a

Note on above study: U.S. EPA/OPP 1997a attributes this study to Roberts and Parke 1976. Roberts and Parke 1976, however, only assayed mallard ducks. A review of the CBI files did not identify an acute oral study in bobwhite quail. The above entry is included in the peer review draft *but should be deleted* in the final report unless the value can be verified.

Species	Nature of Exposure	Exposure Time	Effects	Reference
Red-winged black birds, *Agelaius phoeniceus*, 5 or 6/dose group	single gavage dose of 1000, 2500, 3000, 4000, or 5000 mg/kg bw technical grade (99%) TH 6040; observation period of 14 days	single dose	Mortality: 1/6 at 1000 mg/kg (considered unrelated to treatment); 0/5 at 2500 mg/kg 1/6 at 3000 mg/kg following signs of piloerection, asthenia, and ataxia; 4/6 at 4000 mg/kg 5/6 at 5000 mg/kg NOEC = 2500 mg/kg bw	Alsager and Cook 1975 MRID 00038614

Acute Dietary

Species	Nature of Exposure	Exposure Time	Effects	Reference
Mallard ducks	in diet concentrations ≤4640 ppm technical grade TH-6040 (purity assumed to be 100%) dissolved in corn oil	8 days	NOEC =4640 ppm; no mortality and no observable signs of toxicity.	Fink and Petrocelli 1973 MRID 00038613

Appendix 4: Toxicity of diflubenzuron and diflubenzuron formulations to birds

Species	Nature of Exposure	Exposure Time	Effects	Reference

Reproduction Studies

Species	Nature of Exposure	Exposure Time	Effects	Reference
Mallard ducks, *Anas platyrhynchos*, young adults, 16/sex/dose group	dietary nominal concentrations of 0, 250, 500, or 1000 ppm. Based on mean body weights (about 1.25 kg) and mean food consumption (about 160 g/day), the dietary concentrations correspond to about 0, 32, 64, and 128 mg/kg bw/day.	20 weeks	No treatment-related mortality; no overt signs of toxicity; no treatment-related effects on body weight or feed consumption; no treatment-related effects of reproduction; and no treatment-related effects on body weights of hatchlings or 14-day old survivors.. At 1000 ppm, there was slight, but statistically significant decrease in mean egg shell thickness. NOEC = 500 ppm	Beavers et al. 1990a MRID 41668001
Bobwhite quail, *Colinus virginianus*, young adults, 16/sex/dose group	dietary nominal concentrations of 0, 250, 500, or 1000 ppm. Based on mean body weights (about 200 g) and mean food consumption (about 22 g/day), the dietary concentrations correspond to about 0, 27.5, 55, and 110 mg/kg bw/day.	21 weeks (1-generation)	No treatment-related mortality, overt signs of toxicity, or effects on body weight or food consumption during experimental period. At 1000 ppm, there was a marginal decrease in the number of eggs laid. NOEC (based on possible effect on egg production at 1000 ppm) =500 ppm.	Beavers et al. 1990b MRID 41668002 Beavers et al. 1990c

Appendix 4: Toxicity of diflubenzuron and diflubenzuron formulations to birds

Species	Nature of Exposure	Exposure Time	Effects	Reference
Bobwhite quail, *Colinus virginianus*, adults	dietary nominal concentrations of 2.5, 25, or 250 ppm *air-milled* (99.9% pure) diflubenzuron	12 weeks	No adverse effects on the reproductive parameters measured, including eggs laid, cracked eggs, eggs set, fertile eggs, hatched eggs, egg shell thickness, feed consumption, adult deaths, or chick survival. NOEC = 250 ppm based on review by U.S. EPA/OPP 1997a. The study authors attribute some observed differences between treated groups and controls to random variation and the large sample size (i.e, 500 eggs).	Booth et al. 1977 MRID 00099719
Chickens, White leghorn laying hens, 27-weeks old 10/dose group	dietary nominal concentrations of 0, 10, 50, 100, or 500 ppm diflubenzuron	8 weeks	No adverse effects on food consumption, body weight, egg production, egg weight, egg shell thickness, fertility, hatchability, or progeny development. Diflubenzuron accumulated in eggs and body tissues; 5 weeks after treatment, diflubenzuron was not delectable in the egg, liver, fat, or muscle tissues of hens fed any of the dose levels of the compound.	Cecil et al. 1981 MRID 00156781 Cecil et al. 1981 [published in the open literature]
Growing male broiler and layer chickens	Diflubenzuron at dietary concentrations of up to 250 mg/kg feed	from 1 day of age to 98 days	No consistent differences over time on body weight, food consumption, or testes, liver, comb and feet weights.	Kubena 1981
Layer-breed chickens, males and females	diflubenzuron was fed at levels of 0, 2.5, 25 and 250 mg/kg feed	from 1 day of age through a laying cycle	No effects on egg production, egg weight, eggshell weight, fertility, hatchability or progeny.	Kubena 1982

NOS = Not otherwise specified.

Appendix 5: Toxicity of diflubenzuron to terrestrial invertebrates

Species	Exposure	Effects	Reference
House fly(*Musca domestica*) and parasitoid *Muscidifurax raptor*	Dimilin, topical exposure	No effect to eggs or pupae at 10,000 ppm. > 90% mortality to intermediate to late stage larvae at 1.25 to 10 ppm. No effects to parasitoid.	Ables et al. 1975
Gypsy moth predators: lacewing (*Chrysopa carnea*), ladybird beetle (*Hippodamia convergens*), Wasp parasite *Trichograma pretiosum* of bollworm (*Heliothis*)	10 mg on 9-cm filter paper (contact); and 5 ppm sugar-water fed to host.	Lab rearing of hosts on diflubenzuron diets and raising parasites on those eggs. And raised lacewings from topically treated eggs and adults. Negative effects on lacewing and ladybird beetle in lab; egg hatch of beetle returned to normal after 30-40 d.	Ables et al. 1977
Honey bees, *Apis mellifera L.*	Dietary exposure at concentrations of 0.59, 5.9, and 59 mg/kg diet for 10 days. Vehicle: Sugar syrup.	Reduced brood production at the highest concentration. No effect at two lower concentrations.	Barker and Taber 1977
Honey bees, *Apis mellifera L.*	Diflubenzuron (25% WP) formulation (100 ppm a.i.) supplied in water and 60 ppm supplied in sucrose syrup to colonies of honey bees in outdoor cages.	Brood production almost eliminated; treated bees consumed significantly less water and pollen cake and produced significantly less comb, brood, and new workers. Number of eggs increased in treated colonies. No significant differences in survival of treated bees, compared with controls and both treated and untreated colonies built queen cells when the original queen was removed.	Barker and Waller 1978
Rice swarming caterpillar adult *Spodoptera mauritania*	Dimilin 25-WP, dietary exposure	60-64% sterility at 10 ppm, 100% sterility at 100-1,000 ppm	Beevi and Dale1984
Gypsy moth *Lymantria dispar*	topical exposure	LD_{50} = 3.58 mg/kg (alder) LD_{50} = 8.96 mg/kg (douglas fir)	Berry et al. 1993
Gypsy moth *Lymantria dispar*	acute oral exposure	LC_{50} = 0.06 ppm diet (alder) LC_{50} = 0.45 ppm diet (douglas fir)	Berry et al. 1993
earthworm (Eisenia fetida)	soil exposure	NOEC = 1 g Dimilin WP-25 per kg dry soil	Berends and Thus 1992
earthworm (Eisenia fetida)	soil exposure	NOEC = 780 mg diflubenzuron per kg dry soil	Berends et al. 1992

Appendix 5: Toxicity of diflubenzuron to terrestrial invertebrates

Species	Exposure	Effects	Reference
Nontarget insects (lacewing *Chrysopa oculata*, braconid wasp *Macrocentrus ancylivorous*, assassin bug *Acholla multispinosa*)	Dimilin 25-WP - topical exposure up to 300 ppm and contact with treated leaves consumption of treated host larvae	Considerable mortality and inhibition of molting to lacewing, but no effects to wasp or bug. reduced emergence of wasp, but no effect on lacewing.	Broadbent and Pree1984a
cockchafer *Melolontha melolontha*, leaf beetle *Gastroidea viridula*	beech or sorrel leaves treated with 0.1% Dimilin 25-WP	repellant effects and 100% ovicidal effect to chafer. Effective against larvae and eggs of beetle.	Büchi and Jossi1979
Honey bee	Dimilin - topical exposure	LD_{50} = 52.9 mg/kg (3rd instar) LD_{50} = 45.51 mg/kg (4th instar) LD_{50} = 22.33 mg/kg (pupa)	Chandel and Gupta1992
Bee *Apis cerana indica*	Dimilin - topical exposure	LD_{50} = 56.15 mg/kg (3rd instar) LD_{50} = 49.13 mg/kg (4th instar) LD_{50} = 22.69 mg/kg (pupa)	Chandel and Gupta1992
Spined soldier bug, *Podisus maculiventris*, (predator)	Topical, residual, and oral exposure to diflubenzuron 48% suspension concentrate.	Diflubenzuron harmless to predatory bug by direct and residual contact, but highly toxic when ingested via drinking water. Five days after adult emergence, LC_{50} (for ingestion to 5th instar nymphs) = 7.20 µg/mL. Exposure of 5th instars to sublethal concentrations (around LC_{10}) had no adverse effects on reproduction of emerging adults.	De Clercq et al. 1995b
Flower bug, *Orius laevigatus*, predatory bug used as a biological control for thrips. N= 20	5th instar nymphs were exposed to formulated diflubenzuron WP 25 via ingestion of contaminated (saturated) cotton wool plug and residual contact for 3 days.	LC_{50} (residual contact) = 391.1 mg a.i./L (95% CI = 140.5-825.6 mg a.i./L) LC_{50} (ingestion) = 229.9 mg a.i./L (95% CI = 108.0-397.3 mg a.i./L)	Delbeke et al. 1997
Migratory grasshopper *Melanoplus sanguinipes*	Dimilin 25-WP, dietary exposure	LC_{50} = 0.08 ppm (lettuce diet) LC_{50} = 0.1 ppm (wheat seedling diet)	Elliott and Iyer1982
Honey bee	Dimilin - topical or dietary exposure	LD_{50} > 30 µg/bee (topical) LD_{50} > 200 µg Dimilin WP-25 per bee (dietary). No adverse effects at 5.9 ppm.	Gijswijt1978

Appendix 5: Toxicity of diflubenzuron to terrestrial invertebrates

Species	Exposure	Effects	Reference
Rove beetle (*Aleochara bilineata*) and Cabbage maggot (target)	Consumption of cabbage maggot treated with Dimilin 25-WP	No adverse effects on rove beetle. Suppression of egg hatching and larva development of the cabbage maggot *Delia radicum*	Gordon and Cornect1986
Desert locust (*Schistocerca gregaria*)	Dietary exposure	LD_{50} = 886.7 µg AI (2nd instar) LD_{50} = 207.4 µg AI (4th instar) LD_{50} = 325.2 µg AI (5th instar)	Jepson and Yemane1991
Mealworms, *Tenebrio molitor*, adults	10 mg/g technical Diflubenzuron incorporated into the diet (wheat flour) for period of ecysis to 9 days	Treatment quantitatively and qualitatively altered the lipid metabolism during sexual maturation. Fatty acid composition of the ovaries was not affected.	Khebbeb et al. 1997
Gram pod borer, *Helicoverpa armigera* (Lepidotera: Noctuidae) [crop pest] eggs 0-24 and 24-48 hours.	eggs dipped for two minutes in different concentrations (NOS) of a suspension of diflubenzuron in distilled water.	IC_{50} (0-24 hours) = 0.0055 ppm (fiducial limits= 0.007-0.004 ppm) IC_{50} (24-48 hours) = 0.0061 ppm (fiducial limits= 0.01-0.0034 ppm)	Kumar et al. 1994
Honey bee	acute topical exposure	LD_{50} > 100 µg/bee (adult) LD_{50} >0.0125 µg/bee (larva)	Kuijpers1989
Honey bee	acute oral exposure	LD_{50} > 100 µg/bee (adult) LD_{50} > 0.030 µg/bee (larva)	Kuijpers1989
Oxya japonica (Orthoptera)	Dimilin 25-WP, topical exposure	LD_{50} = 0.06 µg per insect or 0.31 mg/kg	Lim and Lee1982
Australian ladybird beetle, *Cryptolaemus montrouzierei* , adults (excellent predator of mealybug species)	200 ppm diflubenzuron on treated surface	No adverse effects on longevity or feeding; however treatment had effects on adult females, yielding only 278 progeny, compared with 419 yielded by controls.	Mani et al. 1997
Gypsy moth	Dimilin 25-WP, dietary exposure at 0.1 mg/kg	100% lethal to larvae	Martinat et al. 1988

Appendix 5: Toxicity of diflubenzuron to terrestrial invertebrates

Species	Exposure	Effects	Reference
Grasshopper, *Poekilocerus pictus*, 2-day-old, virgin females	20 µg/insect Diflubenzuron dissolved in acetone applied on the ventral side of the abdomen.	In few treated females, the abdomen could not come out of the sand after egg laying and mortality occurred in the same position. When the abdomen was stretched back, the normal position was not attained again, which may be attributed to the chitin synthesis inhibiting activity of diflubenzuron. Ovaries of treated females were adversely affected by treatment, which probably accounts for the decrease in reproduction.	Mathur 1998
Mexican bean beetle	Dimilin 25-WP, dietary exposure	LC_{50} = 3.4 ppm (3rd instar)	McWhorter and Shepard 1977
Lacewing, *Chysoperla carnea*, adults <24 hours old	topical application	At a diflubenzuron at dose of 7,000 ng/insect, no mortality among adults; 100% inhibition of egg hatching due to death embryo. At the lowest dose, 75 ng/insect), 32% reduction in egg hatch.	Medina et al. 2002
Lacewing, *Chysoperla carnea*, adults <24 hours old	topical application	LD_{50} = 2.26 ng/insect LD_{10} = 0.74 ng/insect LD_{90} = 6.87 ng/insect No effect on reproduction at a dose of 0.5 ng/insect.	Medina et al. 2003
Honey bees, caged colonies	10 mg/kg diflubenzuron for 10 weeks	No adverse effects on pollen consumption or brood production; however treatment resulted in a 50% decrease in the amount of syrup stored.	Nation et al. 1986
Cotton leafworm *Spodoptera littoralis*	Dietary exposure	LC_{50} = 1 mg/kg	Neumann and Guyer1987
Predacious phytoseiid mite, *Amblyseius womersleyi*, adult females	Diflubenzuron (Dimilin) (25% pure) at field rate of 100 ppm on bean leaf disks dipped in test substance	No mortality 3 days after treatment.	Park et al. 1996
Oncopeltus fasciatus, Large milkweed bug	Topical exposure to 1 µg/insect	Inhibition of reproduction	Redfern et al. 1980

Appendix 5: Toxicity of diflubenzuron to terrestrial invertebrates

Species	Exposure	Effects	Reference
Brown lacewing, *Micromus tasmaniae* (beneficial predator)	contact exposure: 0.07 µg/cm^2 a.i. as Dimilin 25 WP sprayed on petri dishes	Treatment caused a strong trend toward decrease in fertility where 13% of all pairs did not lay any eggs; total numbers of eggs produced per females were reduced by approx. 50%; treated females deposited significantly fewer eggs per day than the control females (p<0.01).	Rumpf et al. 1998
Brown lacewing, *Micromus tasmaniae* (beneficial predator)	contact exposure: Dimilin 25 WP sprayed on petri dishes 32 hours after the 2nd larval molt	120 hour LC$_{50}$ = 0.069% a.i. (95% CI: = 0.049-0.107% a.i.) 360 hour LC$_{50}$ = 0.009% a.i. (95% CI: = 0.003-0.012% a.i.)	Rumpf et al. 1997
European earwig *Forficularia auricularia*	12.5 g a.i./ha	growth and mobility adversely affected	Sauphanor et al. 1993
Pieris brassicae (Large White Butterfly)	Topical exposure	LD$_{50}$ = 2.5 µg/insect or 1.07 mg/kg	Sinha et al. 1990
Mealworms, *Tenebrio molitor*, adults	5 or 10 mg/g Diflubenzuron (NOS) incorporated into diet for 3 or 6 days post emergence.	Diflubenzuron had no significant effect on fat body protein.	Soltani-Mazouni and Soltani 1995a
Mealworms, *Tenebrio molitor*, adults	5 or 10 mg/g Diflubenzuron (NOS) incorporated into diet. Duration of exposure not clear.	treatment caused a decrease in both the cell density of germarium and the thickness of chorion.	Soltani and Soltani-Mazouni 1997
Mealybug ladybird beetle, *Crptolaemus montrouzieri*, predator of mealybugs	freshly emerged final instar nymphs were fed with mealy bugs treated with 0.153 ppm Diflubenzuron and sacrificed after 24, 48, 72, or 96 hours.	There was a significant reduction in protein content after 2 hours; however, with prolonged exposure, the insect was found to adapt itself to the toxic stress and the adverse effect was much less pronounced after 96 hours.	Sundari et al. 1998
Honey bee	oral and contact LD$_{50}$ values	>30 µg/bee	Stevenson 1978

Appendix 5: Toxicity of diflubenzuron to terrestrial invertebrates

Species	Exposure	Effects	Reference
Honey bee, *Apis mellifera*	0.1, 1, & 10 ppm in Sugar-cake for 12 wks. 0.01, 0.1, & 1.0 ppm in sucrose syrup next year for 10 weeks.	At 10 ppm diflubenzuron in sugar-cake, significantly fewer sealed brood were produced, and colony size was reduced significantly compared to control and lower dosed colonies. No effects on brood production, colony size or adult bee mortality were seen the following year, when lower doses in a fluid solution was used. Degradation in sucrose solution might have reduced the potential for adverse effects.	Stoner and Wilson 1982
Fruit-sucking moth, *Othreis materna*, 5th instar larvae	topical application of 0 or 0.025 µL Dimilin (25 WP) in 5 µL acetone to ventral region of the abdomen. Larvae were sacrificed 24, 48, or 72 hours after exposure.	Inhibition of molting in larvae seems to occur due to neuroendocrine failure. See Section 4.1.2.3. for discussion.	Tembhare and Shinde 1998
Honey bee colonies	Diflubenzuron diluted with sucrose to a rate equivalent to maximum application rate on flowering crops.	Treatment with diflubenzuron resulted in short-term decrease in the numbers of adult bees and brood, compared with controls. No significant effect on development of brood during the following spring; however, there appeared to be a slower expansion, compared with controls. No adverse effects on queen viability.	Thompson and Wilkins 2003
Nematodes	10 day dietary exposure to Dimilin at 10 ppm	Adults unaffected but reproduction hindered and egg hatch prevented. Population reductions of 5% for *Pelodera* sp., 47% for *Panagrellus redivivus*, and 94% for *Acrobeloides* sp.	Veech 1978
German cockroach *Blatella germanica*	Dimilin 25W® - contact with spray of treated cage plywood panels	population reduction of 67.3% at 30 mg/m^2, 93% at 60 mg/m^2, and 98.2% at 120 mg/m^2. egg hatch unaffected, but high first instar mortality.	Wadleigh et al.1991
Codling moth (*Cydia pomonella*), neonates of field-collected and laboratory strains	Dimilin WP	5-day LC$_{50}$ = 13.9 mg/L (95% CI = 10.7-18.2 mg/L)	Weiland 2000 MRID 45245403

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Appendix 5: Toxicity of diflubenzuron to terrestrial invertebrates

Species	Exposure	Effects	Reference
Honey bee	Dimilin 25-WP, dietary	$LC_{50} = 3.7$ ppm	Wittmann1982
Honey bee	Diflubenzuron dietary	No toxicity at concentrations up to 1000 mg/kg in the diet.	Yu et al. 1984
Stinkbug, *Podisus nigrispinus*, eggs and nymphs	Diflubenzuron sprayed on eggs and nymphs.	No effect on egg viability.	Zacarias et al. 1998
Host: Mexican bean beetle (*Epilachna varivestis*). Parasite: wasp (*Pediobius foveolatus*).	100, 1,000, and 10,000 ppm	Topical application to adults did not affect survival or reproduction, nor that of their progeny. Emergence of parasite from larvae treated after parasitism and before was 0 or nearly 0.	Zungoli et al. 1983

Appendix 6: Toxicity of diflubenzuron to fish

Species	Nature of Exposure	Exposure Time	Effects[a]	Reference
		Diflubenzuron		
Acute				
Bluegill sunfish, *Lepomis macrochirus*	static renewal bioassay	96 hours	LC_{50} = 135 mg/L	Marshall and Hieb 1973 MRID 00056150
Fathead minnow	static	96 hours	LC_{50} > 500 mg/L	Reiner and Parke 1975 MRID 00060376
Mummichog, *Fundulus heteroclitus*	static renewal bioassay	96 hours	NOEC = 29.86 mg/L LC_{50} = 32.99 (CL = 29.01-37.52 mg/L)	Lee and Scott 1989
Rainbow trout, *Salmo gairdneri*	static renewal bioassay	96 hours	LC_{50} = 140 mg/L	Marshall and Hieb 1973 MRID 00056150
Rainbow trout, Channel Catfish, and Bluegills	static	96 hours	LC_{50} > 100 mg/L	Johnson and Finley 1980
Brook trout	static	96 hours	LC_{50} > 50 mg/L	Johnson and Finley 1980
Yellow perch	static	96 hours	LC_{50} = 25 mg/L	Johnson and Finley 1980
Rainbow trout	static	96 hours	LC_{50} = 240 mg/L as Dimilin 25-WP	Julin and Sanders 1978
Channel catfish	static	96 hours	LC_{50} = 370 mg/L as Dimilin 25-WP	Julin and Sanders 1978
Fathead minnow	static	96 hours	LC_{50} = 430 mg/L as Dimilin 25-WP	Julin and Sanders 1978
Bluegill sunfish	static	96 hours	LC_{50} = 660 mg/L as Dimilin 25-WP	Julin and Sanders 1978
Yellow perch	static	96 hours	LC_{50} > 50 mg/L	Mayer and Ellersieck, 1986
Brook trout	static	96 hours	LC_{50} > 50 mg/L	Mayer and Ellersieck, 1986
Cutthroat trout	static	96 hours	LC_{50} > 60 mg/L	Mayer and Ellersieck, 1986
Atlantic salmon	static	96 hours	LC_{50} > 50 mg/L	Mayer and Ellersieck, 1986

Appendix 6: Toxicity of diflubenzuron to fish

Species	Nature of Exposure	Exposure Time	Effects[a]	Reference
Longer Term				
Fathead minnows	continuous exposure to concentrations of 0, 0.00625, 0.0125, 0.025, 0.05, or 0.10 ppm 99.4% pure TH-6040 (air milled)	10 months	No effects on survival, growth, behavior or reproduction, compared with controls; no observable effects on hatchability of eggs spawned by fish. Fry, hatched from eggs spawned by treated fish showed no appreciable differences, compared with controls after 60 days of exposure to TH-6040, under same conditions as parental fish.	Cannon and Krize 1976 MRID 00099755
Salmonids (steelhead trout) and non-salmonids (fathead minnows and guppies) fish species	Diflubenzuron under flow-through conditions at concentrations up to 45 µg/L.	96 hours or 30 days (survival and growth in early life stages	No effects at any concentration. NOEC >45 µg/L (highest concentration tested)	Hansen and Garton 1982a
Mummichug, *Fundulus heteroclitus* (marine species)	Life cycle involving continuous (flow through) exposure to TH-6040 dissolved in acetone to deliver concentrations of 0.003, 0.006, 0.0125, 0.025, or 0.05 ppm	life cycle (2-generations)	No significant dose-response relationships.	Livingston and Koenig 1977 MRID 014402120 Livingston and Koenig 1977 MRID 00099722
Mesocosm				
Bluegill sunfish, *Lepomis macrochirus*, "young-of-the year"	Dimilin at nominal treatment levels of 0.7, 2.5, 7.0, or 30 µg/L to littoral enclosures	70 days	NOEC = 0.7 µg/L LOEC = 2.5 µg/L Secondary effects on endpoints based on growth (individual fish size). See additional notes below.	Moffett and Tanner 1995 *In*: Moffett 1995 MRID 44386201

Species	Nature of Exposure	Exposure Time	Effects[a]	Reference

Additional Notes on Moffett and Tanner 1995: In indigenous fish species, mean fish size, population numbers, and biomass were not affected by exposure to diflubenzuron ($\leq$30 µg/L). Indigenous species included brook stickleback, northern redbelly dace, and central mudminnows. Young-of-the-year bluegill growth rates were directly correlated to the density of several invertebrates (cladoceran and copepods) in the enclosures and inversely correlated to the measured concentration of diflubenzuron. The results indicate that the indirect effects of diflubenzuron on bluegill sunfish were caused by a reduction in food resources due to the direct toxicity of the pesticide on the chitinous invertebrates preferred by the bluegill.

Species	Nature of Exposure	Exposure Time	Effects[a]	Reference
Bluegill sunfish, *Lepomis macrochirus*	Dimilin 25 W in littoral enclosures at nominal concentrations of 2.5 or 30 µg/L	reproductive cycle	Treatment adversely affected reproductive success by decreasing growth of young of the year bluegills at 2.5 and 3.0 µg/L by eliminating or reducing preferred bluegill food choices (cladocerans and copepods).	Tanner and Moffett 1995 *In*: Moffett 1995 MRID 44386201

Additional Notes on Tanner and Moffett 1995: No behavioral effects related to reproduction of adult bluegills were observed in the enclosures. There was no clearly determined effect on spawning; however it appeared by spawning was influenced more by water temperature than by diflubenzuron. No direct effects on larvae prior to swim-up; however secondary effects on growth were evident following swim-up, apparently due to the precipitous decrease of zooplankton and the decline of chironomids and other macroinvertebrates.

Bioconcentration

Species	Nature of Exposure	Exposure Time	Effects[a]	Reference
Bluegill sunfish, *Lepomis macrochirus*	dynamic 42-day study to evaluate bioconcentration of C [14]-diflubenzuron	28 days under flow-through conditions, with 14 day depuration period	In fillet, the BCF was 120 after 1 day and 170 after 28 days with a peak of 200 after 7 days. In whole fish, the BCF was 260 after 1 day and 350 after 28 days with a peak of 360 after 7 days.	Burgess 1989 MRID 42258401
White crappies	10 ppb DFB	24 hours	BCF = 82.2	Schaefer et al. 1979
Bluegill sunfish	10 ppb DFB	24 hours	Residues of approximately 848 ppb; 218 ppb in skin and 232 ppb in inner tissues (NOS); residues decreased rapidly when fish were transferred to the rinse tank for $\geq$48 hours.	Schaefer et al. 1979

Appendix 6: Toxicity of diflubenzuron to fish

Species	Nature of Exposure	Exposure Time	Effects[a]	Reference
p-Chloroaniline				
Acute				
Bluegill *Lepomis macrochirus*	Static	96 hour	LC $_{50}$ value = 2.4 mg/L	WHO 2003
Longer Term				
Medaka, *Oryzias latipes*	Larval growth; flow-through	28 days	MATC <2.25 mg/L	WHO 2003
Zebra fish *Brachydanio rerio*	growth and reproduction at 0.04, 0.2, and 1 mg/liter	5 weeks	Adverse effects at 1 mg/L: abdominal swelling, spinal deformations, reduced number of eggs, and reduced fertilization in the F1 and F2 generations.	Bresch et al. 1990
Zebra fish *Brachydanio rerio*	Flow-through	3 weeks	NOEC for Mortality and other effects = 1.8 mg/L	WHO 2003
Bioconcentration				
Medaka, *Oryzias latipes* (Killifish)	Static aqueous exposures to [^{14}C]-**chloroaniline** (8.9-17 mCi/ mmol; >98% pure) for up to 320 minutes	up to 320 minutes	Due to low elimination rates, 20% of the absorbed dose remained within the fish through 330 minutes after exposure. N-acetylation was the dominant route of *in vivo* metabolism, with no indication of ring hydroxylation.	Bradbury et al. 1993
Carp, *Cyprinus carpio*	continuous flow-through exposure to 0.30±0.07 or 10.4±0.4 µg/L **p-chloroaniline**	up to 335 hours (about 14 days)	average BCF in whole body were 1.7 (low concentration) and 0.8 (high concentration).	Tsuda et al. 1993

[a] Values in parentheses are the 95% confidence limits.

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Grass shrimp, *Palaemonetes pugio*	Subchronic exposure to measured concentrations of 0.70, 1.73, 5.51, 6.79, or 16.4 µg/L for 35 days in flowing seawater	No survival to day 7 among zoea exposed to initial measured concentrations of 5.5, 6.8, or 16.4 µg/L; survival among shrimp exposed to 0.70 or 1.73 µg/L was significantly less than survival among controls; no significant difference in size of shrimp exposed to 0.70 or 1.73 µg/L, compared with controls.	Bionomics-EG&G 1975 MRID 00038612
Grass shrimp, *Palaemonetes pugio*	Acute exposure to nominal concentrations of ≤1.0 mg/L TH-6040 in static seawater	96-hour LC_{50} = 0.64 mg/L (0.13-3.1 mg/L)	Bionomics-EG&G 1975 MRID 00038612
Hydropsychidae (Trichoptera)	Dimilin 25-WP, 15 days at 0.0025 to 0.25 mg/L	No adult emergence from treated tanks and only 31.6% emergence from control tanks	Bradt and Williams 1990
Mysid shrimp, *Mysidopsis bahia*, F_1 second generation	mean measured concentration of 123 ng/L (0.123 µg/L) diflubenzuron (97.6% pure) for up to 5 days	upon removal of treated water, juvenile second generation mysids completely recovered and had survival and reproductive success similar to that of the controls.	Breteler 1987 MRID 40237501
Mysid shrimp,*Mysidopsis bahia*, juvenile	Continuous exposure to mean measured concentrations of 29, 45, 86, 140 or 210 ng/L diflubenzuron through entire life cycle over a 28-day test period. Juvenile mysids produced during the test at the lowest four test concentrations (29-140 ng/L) were continuously exposed for the 8 days of the 28-day test.	F_0 survival at 86, 140, and 210 ng/L was significantly reduced (p≤0.05) compared with controls; treatment caused significant reduction in growth and development (as measured by dry weight) in F_0 males (210 ng/L) and F_0 females (140 and 210 ng/L); reproduction of F_0 mysids was significantly reduced at 86, 140, and 210 ng/L. The NOEC = 86 ng/L for growth LOEC = 140 ng/L for growth. Survival of the second generation (F_1) mysids was not affected by continuous exposure to any of the mean measured concentrations tested (21, 33, 83, or 123 ng/L). The NOEC after 8 days of exposure of F_1 generation mysids was >83 ng/L.	Breteler 1987 MRID 40237501 Note: This summary is of the ***primary study*** on which the studies discussed below are based.

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Mysid shrimp, *Mysidopsis bahia*	24-hour exposure to mean concentration of 298 ng/L diflubenzuron (97.6% pure), followed by transfer to clean control water for 27 days.	Survival, growth, and reproductive success similar to that of controls.	Breteler 1987 MRID 40237501
Marine crabs, *Pontonia pinnophylax*, larvae	≤10 ppb diflubenzuron	larvae of four different crab species appeared normal during inter-molt periods and adverse effects were apparent until molting (similar to effect of DFB on insect larvae). Treatment deformed both the exocuticle and the endoculticle and was lethal to all four species of marine crabs.	Christiansen 1987
Mixed aquatic invertebrates (i.e., cladocerans, rotifers, and adult amphipods)	Microcosm 1: nominal concentrations of 0.3, 0.7, 1.4, 3.4, 6.8, or 13.6 µg/L Dimilin 25W Microcosm 2: nominal concentrations of 1.4. 3.4. 6.8, or 20.0 µg/L Dimilin 25W	Major effect of diflubenzuron in the microcosms was on the cladocerans. Population density was decreased within 3-4 days after treatment at ≥0.7 µg/L and remained consistently low, compared with controls throughout the study duration. Statistically significant ($p \leq 0.05$) differences in population density at ≥1.4 µg/L in Microcosm 1 between days 3 and 10 and at ≥0.7 µg/L in Microcosm 2 between days 4 and 14. Cladoceran population densities did not generally increase in either microcosm at ≥0.7 µg/L. Rotifers were no adversely affected by treatment at any concentration. The numbers of adult amphiphods (*Hyalella azteca*) were significantly different from controls ($p \leq 0.05$) at 13.6 µg/L (Microcosm 1) and 20 µg/L (Microcosm 2). *Amphipods exposed to concentrations <13.6 µg/L were not different ($p \leq 0.05$) from controls in either experiment.* NOEC for cladocerans = 0.3 µg/L LOEC for cladocerans = 0.7 µg/L	Corry et al. 1995 *In*: Moffett 1995 MRID 44386201

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Fiddler crabs, *Uca pugilator*, juveniles	repetitive 24-hours weekly exposures to 0.2, 2, 20, or 200 µg/L Dimilin in static seawater systems for 10 weeks.	NOEC (time to first molt) =20µg/L NOEC (survival) = 2 µg/L NOEC (ability to escape from test container) = 0.2 µg/L Behavioral effect caused by DFB exposure ($\geq$2 µg/L) was most sensitive indicator of DFB toxicity. Investigators conclude that survival, molting, and behavior of juvenile fiddler crabs are significantly affected by exposure to repetitive applications of DFB.	Cunningham and Meyers 1987
Barnacles, *Balanus eburneus*, Cirripede crustaceans.	Exposure to1-1000 µg/L technical grade, air-milled diflubenzuron w/acetone as carrier solvent (preliminary studies showed no mortality in acetone controls) for 28 days	Dose-dependent mortality, with drastic mortality observed during the second week of exposure. Lethal and sublethal effects were observed at concentrations as low as 50 µg/L Disruption of the exoskeleton caused by diflubenzuron was similar to that observed in insects. Development of barnacles exposed to diflubenzuron for 10 days or more at 750 and 1000 µg/L was delayed in the pre-molt phase of cuticle secretion	Gulka et al. 1980
Ceriodaphnia dubia, neonates, <12 hours old	Exposure to 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, or 4.0 ng/mL Dimilin for 48 hours.	48-hr NOEC = 0.75 ng/mL [0.75 µg/L] 48-hr LC_{50} =1.7 ng/mL (95% CI = 1.36-2.02 ng/mL) [1.7 µg/L]	Hall 1986 MRID 40130601
Ceriodaphnia dubia	Chronic exposure to 0, 0.05, 0.1, 0.25, 0.5, 0.75, or 1.0 ng/mL (µg/L). Used methanol carrier with carrier control.	NOEC = 0.25µg/L At $\geq$0.5 µg/L, significant decrease in numbers of neonates produced, compared with controls; at 0.75 and 1.0 µg/L, adults produced no viable young; mortality increased at exposures to >0.1 µg/L. No carrier effect: 31.7 (28.4-34.9) neonates/female with 20% mortality in adults in untreated control and 30.9 (26.9-35) in carrier control with 10% mortality in adults.	Hall 1986 MRID 40130601

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
CRITICAL NOTE on HALL 1986: Hall (1986) reports concentrations as nanograms/mL. These are converted above to µg/L.			
Daphnia magna	Diflubenzuron under static conditions for 48 hours	LC_{50} = 1.84 µg/L (95% CI = 0.05-3.71 µg/L)	Hansen and Garton 1982a
Midges, *Tanytarsus dissimilis*, 2nd to 3rd larval instar	Diflubenzuron under flow-through conditions for 5 days; effect criteria = molting success	LC_{50} = 1.02 µg/L (95% CI = 0.56-1.47 µg/L)	Hansen and Garton 1982a
Midges, *Cricotopus*, sp, 4th larval instar to pupae	Diflubenzuron under flow-through conditions for 7 days; effect criteria = molting success	LC_{50} = 1.79 µg/L (95% CI = 1.48-2.13 µg/L)	Hansen and Garton 1982a
Daphnia magna	Survival and reproduction in full life cycle after exposure to diflubenzuron (conditions not specified)	LC_{50} = 0.062 µg/L (95% CI = 0.051-0.071 µg/L)	Hansen and Garton 1982a
Freshwater molluscs (two species of snails)	Diflubenzuron under flow-through conditions for 96 hours; effect criteria for chronic exposure (3 weeks) = survival, growth and reproduction	NOEC 45 µg/L (highest concentration tested)	Hansen and Garton 1982a
Stream invertebrates (most abundant), including Ephemeroptera, Plecoptera, Diptera, Tricoptera, and Coleoptera.	Technical diflubenzuron in dimethlformamide at 0.1, 1, 10, and 50 µg/L added continuously to complex laboratory stream channels supplied periodically with field-collected microorganisms for 5 months	Invertebrates were most adversely affected undergoing rapid and permanent reductions in biomass and diversity at diflubenzuron concentrations of ≥1.0 µg/L. These effects were the results of major in reductions in many of the aquatic insect populations, primarily among mayflies, stoneflies and diptera.	Hansen and Garton 1982a

Species	Exposure Time	Effects[a]	Reference

Additional Notes on Hansen and Garton 1982a: Diversity in all groups of stream invertebrates was clearly dose-related with little or no reductions observed at 0.1 µg/L, intermediate reductions observed at 1.0 µg/L (some dipteran tax were relatively insensitive at this concentration but eliminated at higher concentrations), and maximal reductions observed at ≥10.0 µg/L.

Algal, fungal, and bacterial functional groups were also adversely affected by exposure to diflubenzuron. Generally the adverse effects observed among these organisms was variable and transient alterations in biomass and diversity with algae and bacteria affected at 1.0 µg/L and fungi affected at as little as 0.1 µg/L.

Species	Exposure Time	Effects[a]	Reference
Total biological community in 8 stream microcosms	8- month continuous exposure to 0.1, 1.0, 10, or 50 µg/L diflubenzuron dissolved in dimethylformamide	Insects were directly affected at ≥1.0 µg/L (stoneflies and mayflies were the most sensitive with adverse effects apparent at 1.0 µg/L, dipterans affected at 10.0 µg/L, and coelopterans were not affected at any test concentrations); Algae and fungi were mildly affected at ≥1.0 µg/L, but the effects were considered indirect in response to the decreases in herbivore and shredder components of the insects; No effects were observed in bacteria, oligochaetes, or gastropods at any test concentration.	Hansen and Garton 1982b
Gammarid, *Hyallela azteca* (Benthic crustacea)	Diflubenzuron under flow-through conditions for 96 hours	$LC_{50} = 1.84$ µg/L (95% CI = 0.05-3.71 µg/L)	Hansen and Garton 1982a
Stoneflies, *Peltoperla arcuata* and *Pteronarcys proteus*	DFB-treated yellow poplar leaves via ingestion for 24-hours with 60- and 90-day observation periods.	*Peltoperla*: survival significantly different from controls at day 60; however survival of *Pteronarcys* was not significantly different from controls at 90 days, although the low number of molts that occurred during that time may have influenced the results.	Harrahy et al. 1994

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Stoneflies, *Peltoperla arcuata*	nominal concentrations of 0, 1.0, 10, 100, or 1000 ppb DFB in dechlorinated tap water for 96 hours and then transferred to glass chambers containing pesticide-free water and fed stream conditioned red maple and white oak leaves.	Survival at 10 and 1000 ppb was significantly different from controls; however, survival at 100 ppb was not significantly different from survival of controls. No behavioral changes were observed.	Harrahy et al. 1994
Mayflies, *Cyngmula subaequalis*, *Stenacron interpunctatum*, *Stenonema meririvulanum*, and *S. femaratum*	0, 0.6, 5.6, 55.7, or 557.2 ppb DFB (Dimilin 25% WP) in water for 96 hours then placed in pesticide-free water for 36-day observation period	after 4 days of exposure, mayflies were significantly lower than controls al all concentrations tested. At the lowest concentration, only about 45% survived to day 36. Many of the treated mayflies died while molting, while others died from incomplete hardening of the new cuticle. Behavioral changes observed included decreased swimming speed at higher concentrations, and no avoidance of pipet or hands during water replacement activities. Some mayflies were observed to shake sporadically before dying.	Harrahy et al. 1994
Daphnids, *Daphnia magna*	48-hour exposure to diflubenzuron (97.6% pure)	48-hour NOEC = 0.45 µg/L 48-hour EC_{50} = 7.1 µg/L (95% CI = 5.0-1.0 µg/L)	Kuijpers 1988 MRID 40840502
Fairy shrimp, *Streptocephalus sudanicus*, females	Dimilin (solvent-based, liquid ULV formulation) for 24 or 48 hours under static conditions	24-hour EC_{50} =13.3 µg/L (range = 12.8-14.0 µg/L) 48-hour EC_{50} =0.74 µg/L (range = 0.60-0.88 µg/L)	Lahr et al. 2001
Backswimmer, *Anisops sardeus*, females	Dimilin (solvent-based, liquid ULV formulation) for 24 or 48 hours under static conditions	24-hour EC_{50} =2123 µg/L (range = µg/L) 48-hour EC_{50} =1937 µg/L (range = 1800-2020 µg/L)	Lahr et al. 2001
Daphnia magna	Technical grade diflubenzuron (TH-6040)	LOEC for reproduction: 0.09 ppb	LeBlanc 1975

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Blue crabs, *Callinectes sapdidus*, embryos	acute toxicity; diflubenzuron exposure in culture plates	hatching EC_{50} =1.8 µg/L	Lee and Oshima 1998
Littoral enclosure community of mixed insects	Dimilin at nominal treatment levels of 0.7, 2.5, 7.0, or 30 µg/L to littoral enclosures	EC_{50} = 1.2 µg/L (measured concentration) NOEC = 1.0 µg/L (measured concentration) LOEC = 1.9 µg/L (measured concentration)	Liber 1995 *In*: Moffett 1995 MRID 44386201
Littoral zooplankton community dominated by cladocera, copepoda, rotifera, and ostracoda.	Dimilin at nominal treatment levels of 0.7, 2.5, 7.0, or 30 µg/L to littoral enclosures.	Cladocera were extremely sensitive to treatment, with mean population abundances significantly reduced, compared with controls, at all four treatment levels. Mean population densities at ≥2.5 µg/L were 92 to >99% lower than mean control values by day 6 and remained at those levels through day 56. None of the decreased populations at ≥2.5 µg/L showed any sign of recovery throughout the study. Copepoda were adversely affected by treatment at all concentration levels. LOEC = 0.7 µg/L. The measured peak diflubenzuron concentration in water was 1.0 µg/L. Copepoda were significantly affected at this level, not unlike the Cladocera. The NOEC for both Claodcera and Copepoda was defined as <0.7 µg/L; however the effects at 0.7 µg/L appeared to be transistory with recovery after a single application observed within 12-29 days. Ostracoda densities were reduced at the two highest concentrations. NOEC = 2.5 µg/L Rotifera were not affected by treatment at any concentration level. NOEC = >30 µg/L.	Liber and O'Halloran 1995 *In*: Moffett 1995 MRID 44386201 Published as Liber et al. 1996 and as O'Halloran et al. 1996
Chironomus plumosus, 4th instar larvae	Dimilin 25-WP, 48 hour exposure	EC_{50} = 0.56 mg/L	Julin and Sanders 1978

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Daphnia magna	Dimilin 25-WP® - 48 hour exposure	LC_{50} = 0.00075 mg/L (neonate) LC_{50} = 0.02345 mg/L (adult)	Majori et al. 1984
Dragonfly nymphs *Orthemis* spp., *Pantala* sp.	TH 6040 (diflubenzuron) - 168 hour exposure	LC_{50} = 50 µg/L	Miura and Takahashi 1974
Mayfly nymphs *Callibaetis* sp.	TH 6040 (diflubenzuron) - 168 hour exposure	LC_{90} = 10 µg/L	Miura and Takahashi 1974
Aedes nigromaculatum	TH 6040® (diflubenzuron) - 48 hour exposure	LC_{50} = 0.5 µg/L	Miura and Takahashi 1974
Water scavenger beetle larvae *Hydrophilus triangularis*	TH 6040® (diflubenzuron) - 48 hour exposure	LC_{50} = 100 µg/L	Miura and Takahashi 1974
Water scavenger beetle adults *Laccophilus* spp., *Thermonectus basillaris*, *Tropisternus lateralis*	TH 6040® (diflubenzuron) concentrations as high as 250 µg/L	no mortality	Miura and Takahashi 1974
Mysid shrimp,*Mysidopsis bahia*	life-cycle exposure under flow-through conditions	96-hour LC_{50} = 2.1 µg/L 21-day LC_{50} = 1.24 µg/L direct adverse effect on reproduction: the numbers of juveniles/female were significantly depressed at all nominal concentrations (0.075-0.75 µg/L)	Nimmo et al. 1979
Littoral enclosure community of mixed benthic marcroinvertebrates,predominantly, Chironomidae (midges), Oligochaeta (earthworms), and Mollusca	Dimilin at nominal treatment levels of 0.7, 2.5, 7.0, or 30 µg/L to littoral enclosures. Study duration = 71 days.	Reductions in abundance of Ephemeroptera (mayflies) and Odonata (damselflies and dragonflies) were observed at all nominal concentrations ≥2.5 µg/L. No adverse effects were observed on molluscs or earthworms at any of the four diflubenzuron test concentrations. Overall, the only benthic macroinvertebrate group that appeared to have been adversely affected by exposure to diflubenzuron was the Insecta.	O'Halloran and Liber 1995 *In*: Moffett 1995 MRID 44386201

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Stoneflies (*Pteronarcys proteus* and *Pteronarcys arcuata*)	fed leaves from treated poplar after conditioning in stream	No effect on survival.	Perry 1995a
Blue crabs, *Callinectes sapidus*, juveniles	Dimilin WP-25 in static renewal tests	both molt stage and renewal frequency affected toxicity: LC_{50} (random molt stages) = 3.5 mg/L LC_{50} (day of molt) = 300 µg/L LC_{50} (day of molt and repeated dosing) = 18.5 µg/L	Rebach 1996
Copepods, *Eurytemora affinis*, naupli	0.78 µg/L WP25 commercial DFB (25% DFB, 75% kaolin) and filtered river water for 5 or 6 days	0% survival at >1.69 µg/L; at 0.93 µg/L survival did not differ significantly from controls.	Savitz et al. 1994
Copepods, *Eurytemora affinis*, naupli	WP25 commercial DFB (25% DFB, 75% kaolin) and filtered river water.	48-hour LC_{50} =2.2 µg/L	Savitz et al. 1994
Daphnids, *Daphnia magna*	Continuous exposure to [14]-C-diflubenzuron nominal concentrations of 6.3-100 ng/L (mean measured concentrations of 5.6, 14, 23, 40, or 93 ng/L) under flow-through conditions for 21 days (one generation)	50% survival at 93 ng/L [0.093 µg/L]; survival at the other test concentrations ranged from 93 to 98%, comparable to controls. significant reduction in reproduction and body length at 93 ng/L, compared with controls (p≤0.05); at other test concentrations, reproduction and growth were comparable to controls. NOEC = 40 ng/L [0.04µg/L]	Surprenant 1988 MRID 40840501
Quahog clams, *Mercenaria mercenaria*	48-hour exposure to nominal concentrations of 100 or 500 µg a.i./L (mean measured concentrations of 79, or 320 µg a.i./L) of diflubenzuron (97.6% pure)	No adverse effects on development of quahog embryos and larvae NOEC >320 µg a.i./L	Surprenant 1989 MRID 41392001

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Grass shrimp, *Palaemonetes pugio*	continuous exposure to 1-10 µg/L from inter-molt to molt (normally 7-14 days) and transfer to filtered seawater	Mortalities generally related to molt cycle with death occurring at the time of ecdysis or immediately after (LC_{50} = 0.65 µg/L); at concentrations of 7.5-10 µg/L, some shrimp did not die during the exposure period and displayed delayed progress in the molt cycle, and although these shrimp began progressing through the molt cycle when transferred to filtered seawater, they all failed to reach ecdysis and eventually died. Control shrimp were never observed in an arrested stage in the molt cycle during the experiment.	Tourat and Rao 1987 *In*: Technology Sciences Group 1998 MRID 44399307
Grass shrimp, *Palaemonetes pugio*	24-hour pulsed exposure with transfer to DFB-free medium	LC_{50} =3.4 µg/L (premolt animals D_1 - D_2)	Tourat and Rao 1987 *In*: Technology Sciences Group 1998 MRID 44399307
Grass shrimp, *Palaemonetes pugio*,	96 hours	LC_{50} =1.1 µg/L (premolt animals D_1 - D_2) very few or no mortalities among shrimp in very late premolt, early premolt, intermolt, or early postmolt stages during the 96-hour exposure.	Tourat and Rao 1987 *In*: Technology Sciences Group 1998 MRID 44399307
Horseshoe crabs, *Limulus polyphemus*, eggs	0, 5, or 50 µg/L DFB	at 5 µg/L, crabs showed a slight, but significant ($p<0.05$) delay in molt at 14 days, then molted at a rate comparable to controls and did not exhibit significant mortality. At 50 µg/L, molted at the same rate as controls but exhibited significant mortality immediately after ecdysis. Also, the prosomal width of the crabs in this group was smaller, compared with controls and crabs in the low dose group.	Weis and Ma 1987
snail *Physa* sp.	acute exposure	LC_{50} > 125 ppm	Wilcox and Coffey 1978

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Grass shrimp, *Palaemonetes pugio*, ovigerous carrying 0.5-, 1-,3-, 6-, or 8-day old embryos	continuous exposure for 4 days to 0.3-5.0 µg/L DFB in static system with transfer after exposure to DFB-free seawater for rest of the embryonic development.	No correlation between age of the embryos at exposure and either hatchability or duration of larval development; severity of abnormality did not vary with the age of the embryos except at exposure concentration of 2.5 µg/L. Larval viability was significantly ($p<0.05$) affected by the age of the embryos at the time of exposure to DFB, with older embryos more sensitive to sublethal effects of DFB.	Wilson 1997b
Grass shrimp, *Palaemonetes pugio* at different life stages (embryos, larvae, postlarvae male and female non-spawning adults, and ovigerous females.	96 hours under static renewal conditions	larvae and post-larvae most sensitive to acute toxicity of DFB with LC_{50} values of 1.44 and 1.62 µg/L, respectively; ovigerous females (hence embryos) appeared to be the most resistant to the acute toxicity of DFB with a mean LC_{50} of 6985 µg/L.	Wilson and Costlow 1987
Grass shrimp, *Palaemonetes pugio*	chronic exposure to either technical grade DFB (98.4% a.i.) Or the wettable powder (WP-25) (25% a.i.)	72-hr and 96-hr calculated LC_{50} values were similar for the two formulations of DFB (WP-25 and TG): 72-hr LC_{50} = 2.95 µg/L (TG) 72-hr LC_{50} = 2.83 µg/L (WP-25) 96-hr LC_{50} = 1.84 µg/L (TG) 96-hr LC_{50} = 1.39 µg/L (WP-25) The investigators conclude that results from studies using technical grade DFB are applicable to the WP-25 formulation without the need for a "correction factor."	Wilson and Costlow 1986

Appendix 7: Toxicity of diflubenzuron to aquatic invertebrates

Species	Exposure Time	Effects[a]	Reference
Copepods, *Eurytemora affinis*, naupli, 24- to 48-hours old, initially	0, 0.5, 0.78, or 0.93 ppb DFB under pulse (two 6.5 exposure periods) and continuous (14-day) exposure regimens.	In pulse exposures, copepods exposed in the first 6.5 days showed a significantly lower survival rate at 0.78 and 0.93 ppb; copepods exposed during the second half of the experiment showed no significant differences in mortality, compared with controls. In the 14-day continuous exposure, survival was significantly lower at 0.78 and 0.93 ppb, but was significantly higher than that in the early pulse exposure to 0.78ppb. Effects on brood production were observed at 0.8 ppb in individuals exposed only during the copepodite stages. Significant effects on production of naupli were observed only in the first 6.5 days of pulse exposure to 0.93 ppb. At salinities of 2, 10, and 15 ppt, survival from naupilar to adult stages was significantly reduced at 0.84 ppb and none survived to adulthood at 1.7 ppb.	Wright et al. 1996

[a]Values in parentheses are 95% confidence limits.

Appendix 8: Toxicity of diflubenzuron to aquatic plants

Species	Exposure	Effects [a]	Reference
ALGAE			
Phytoplankton communities in littoral enclosures	Dimilin at nominal treatment levels of 0.7, 2.5, 7.0, or 30 µg/L to littoral enclosures	Phytoplankton, as measured by cell size distributions and chlorophyll *a* in the enclosures, were not affected directly or indirectly by diflubenzuron treatment. No occasions of significant ($p \leq 0.05$) linear correlations between the nominal concentrations of diflubenzuron and phytoplankton measures. These results were consistent with the idea that diflubenzuron does not directly inhibit non-chitinous biota due to the specificity of its mode of action.	Moffett 1995 *In*: Moffett 1995 MRID 44386201
Periphyton communities in littoral enclosures	Dimilin at nominal treatment levels of 0.7, 2.5, 7.0, or 30 µg/L to littoral enclosures	Late in the season (September), a 80 and 90% reduction in periphyton dry weight and 75 and 80% reduction in chlorophyll *a* at 7.0 and 30 µg/L treatment levels, respectively. Differences were statistically significant ($p=0.01$) on day 55 and nearly significant ($p=0.07$) on day 67.	Moffett 1995 *In*: Moffett 1995 MRID 44386201
Macrophyte populations in littoral enclosures	Dimilin at nominal treatment levels of 0.7, 2.5, 7.0, or 30 µg/L to littoral enclosures	No adverse effects, direct or indirect, were observed on macrophyte species composition or total standing crop. There was no correlation between treatment concentrations and total macrophyte density throughout the study. The investigator indicates that direct effects were not anticipated because macrophytes do not have chitin.	Moffett 1995 *In*: Moffett 1995 MRID 44386201
Blue-green algae, *Plectonema boryanum*	0.1 ppm TH-6040 in pure culture for 4 days	No growth inhibition, rapid metabolism of compound in water. Algae degraded 80T of compound in 1-hour incubation period to p-chlorophenyl urea and p-chloroaniline.	Booth and Ferrell 1977

Appendix 8: Toxicity of diflubenzuron to aquatic plants

Species	Exposure	Effects [a]	Reference
Freshwater algae *Selenastrum capricornutum*	300 µg/L diflubenzuron for 5 days	NOEC = 300 µg/L	Thompson and Swigert 1993b MRID 42940104
Freshwater algae, *Selenastrum capricornutum*	120 hour exposures; effect criteria = growth	NOEC 45 µg/L (highest concentration tested)	Hansen and Garton 1982a
Freshwater diatoms (*Navicula pelliculosa*)	380 µg/L for 5 days	NOEC = 380 µg/L	Thompson and Swigert 1993c MRID 42940105
Marine diatoms (*Skeletonema costatum*)	270 µg/L for 5 days	NOEC = 270 µg/L	Thompson and Swigert 1993d MRID 42940106

MACROPHYTES

Species	Exposure	Effects [a]	Reference
Macrophyte populations in littoral enclosures	Dimilin at nominal treatment levels of 0.7, 2.5, 7.0, or 30 µg/L to littoral enclosures	No adverse effects, direct or indirect, were observed on macrophyte species composition or total standing crop. There was no correlation between treatment concentrations and total macrophyte density throughout the study.	Moffett 1995 *In*: Moffett 1995 MRID 44386201
Duckweed (*Lemna gibba*)	190 µg/L diflubenzuron for 14 days	NOEL = 190 µg/L	Thompson and Swigert 1993a MRID 42940103

Pesticide Precautionary Statement

This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife--if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

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Gypsy Moth Management in the United States:
a cooperative approach

Draft
Supplemental Environmental
Impact Statement

Risk Assessments and
Risk Comparison
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Gypsy Moth Management in the United States: a cooperative approach
Draft Supplemental Environmental Impact Statement

The complete Draft Supplemental Environmental Impact Statement, Gypsy Moth Management in the United States: a cooperative approach, consists of four volumes:

Volume I   Summary
Volume II   Chapters 1-8 and Appendixes A, B, C, D, E
Volume III   Appendixes F, G, H, I
Volume IV   Appendixes J, K, L, M

Lead Agency: Forest Service, U.S. Department of Agriculture (USDA)

Responsible Official: James R. Hubbard, Deputy Chief for State and Private Forestry
Sidney R. Yates Federal Building
201 14th Street, SW
Washington, DC 20250

For More Information: Bill Oldland, SEIS Team Leader
180 Canfield Street
Morgantown, WV 26505

Joint Lead Agency: Animal and Plant Health Inspection Service (APHIS), USDA

Responsible Official: Richard L. Dunkle, Deputy Administrator for Plant Protection and Quarantine
4700 River Road
Riverdale, MD 20737-1236

For More Information: Weyman P. Fussell, Ph.D., Gypsy Moth Program Coordinator
Emergency and Domestic Programs, APHIS
4700 River Road, Unit 134
Riverdale, MD 20737-1236

Abstract: The USDA Forest Service and Animal and Plant Health Inspection Service are proposing an addition to the gypsy moth management program that was described in the 1995 Environmental Impact Statement--Gypsy Moth Management in the United States: a cooperative approach--and chosen in the 1996 Record of Decision. The agencies are proposing these new treatment options: adding the insecticide tebufenozide, or adding the insecticide tebufenozide and other new treatment(s) that may become available in the future to manage gypsy moths, provided that the other treatment(s) poses no greater risk to human health and nontarget organisms than are disclosed in this Draft SEIS for the currently approved treatments and tebufenozide.

Commenting on this Draft Supplemental Environmental Impact Statement: Reviewers should provide the Forest Service with their comments during the review period of this draft supplemental environmental impact statement. Timely comments will enable the Forest Service to analyze and respond to all of the comments at one time and to use information acquired in the preparation of the final supplemental environmental impact statement, thus avoiding undue delay in the decision making process. Furthermore, the more specific and substantive the comments, the better for reviewers and the agencies alike. Reviewers have an obligation to structure their participation in the National Environmental Policy Act process so that it is meaningful and alerts the agency to the reviewer's position and contentions (Vermont Yankee Nuclear Power Corp. v. NRDC, 435 U.S. 519, 553, 1978). Environmental objections that could have been raised at the draft stage may therefore be forfeited, if not raised until after completion of the final environmental impact statement (Department of Transportation v. Public Citizen, 541 U.S. 752, 764 (2004). Comments on this draft supplemental environmental impact statement should be specific and should address the adequacy of the statement and the merits of the alternatives discussed (40 CFR 1503.3).

Web Site for Draft SEIS: The Draft SEIS is available for viewing at www.na.fs.fed.us/wv/eis

Send Comments to: Bill Oldland, SEIS Team Leader
USDA Forest Service
180 Canfield Street
Morgantown, WV 26505
304-285-1585

Date Comments Must Be Received: See cover letter for the date that comments are due in Morgantown, WV.

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

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Submitted to:
Dave Thomas, COTR
Forest Health Protection Staff
USDA Forest Service
Rosslyn Plaza Building C, Room 7129C
1601 North Kent Street
Arlington, VA 22209

Prepared by:
Patrick R. Durkin and Julie Klotzbach
Syracuse Environmental Research Associates, Inc.
5100 Highbridge St., 42C
Fayetteville, New York 13066-0950
Telephone: (315) 637-9560
Fax: (315) 637-0445
E-Mail: SERA_INC@msn.com
Home Page: www.sera-inc.com

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                     Risk Assessments, SERA EXWS 04-43-05-06c, Version 3.01.
                     Located at: http://www.fs.fed.us/foresthealth/pesticide/risk.shtml

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AEL	adverse-effect level
a.i.	active ingredient
BCF	bioconcentration factor
bw	body weight
CBI	confidential business information
CI	confidence interval
cm	centimeter
CNS	central nervous system
DAA	days after application
DAT	days after treatment
d.f.	degrees of freedom
EC_x	concentration causing X% inhibition of a process
EC_{25}	concentration causing 25% inhibition of a process
EC_{50}	concentration causing 50% inhibition of a process
ExToxNet	Extension Toxicology Network
F	female
FH	Forest Health
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FOIA	Freedom of Information Act
FQPA	Food Quality Protection Act
g	gram
ha	hectare
HQ	hazard quotient
IAA	indole-3-acetic acid
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
k_a	absorption coefficient
k_e	elimination coefficient
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
$K_{o/w}$	octanol-water partition coefficient
K_p	skin permeability coefficient
L	liter
lb	pound
LC_{50}	lethal concentration, 50% kill
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level
LOC	level of concern
m	meter

M	male
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
mM	millimole
MRID	Master Record Identification Number
MSDS	material safety data sheet
MW	molecular weight
NCAP	Northwest Coalition for Alternatives to Pesticides
NCI	National Cancer Institute
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOS	not otherwise specified
NRC	National Research Council
NTP	National Toxicology Program
OM	organic matter
OPP	Office of Pesticide Programs
OPPTS	Office of Pesticide Planning and Toxic Substances
OSHA	Occupational Safety and Health Administration
ppm	parts per million
RBC	red blood cells
RED	re-registration eligibility decision
RfD	reference dose
SERA	Syracuse Environmental Research Associates
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SRC	Syracuse Research Corporation
UF	uncertainty factor
U.S.	United States
USDA	U.S. Department of Agriculture
U.S. EPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WCR	water contamination rate
WHO	World Health Organization
μ	micron

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

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

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

CONVERSION OF SCIENTIFIC NOTATION

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

EXECUTIVE SUMMARY

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

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

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

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

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

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

HUMAN HEALTH RISK ASSESSMENT

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

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

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

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

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

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

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

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

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

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

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

ECOLOGICAL RISK ASSESSMENT

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

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

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

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

microorganisms have not been noted.

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

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

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

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

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

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

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

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

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

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

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

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

1. INTRODUCTION

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

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

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

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

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

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

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

2. PROGRAM DESCRIPTION

2.1. OVERVIEW

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

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

2.2. CHEMICAL DESCRIPTION AND COMMERCIAL FORMULATIONS

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

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

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

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

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

2.3. APPLICATION METHODS

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

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

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

In some instances, directed foliar applications may be used. In selective foliar applications, the sprayer or container containing the pesticide is carried by backpack and is applied to selected target vegetation. Application crews may treat up to shoulder high brush, which means that chemical contact with the arms, hands, or face is plausible. To reduce the likelihood of significant exposure, application crews are directed not to walk through treated vegetation. Usually, a worker treats approximately 0.5 acres/hour with a plausible range of 0.25-1.0 acre/hour.

In aerial applications, Mimic is applied under pressure through specially designed spray nozzles and booms. The nozzles are designed to minimize turbulence and maintain a large droplet size, both of which contribute to a reduction in spray drift. In aerial applications, approximately 10 acres may be treated per minute (Reardon 2000).

2.4.   MIXING AND APPLICATION RATES

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

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

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

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

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

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

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

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

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

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

3. HUMAN HEALTH RISK ASSESSMENT

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

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

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

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

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

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

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

3.1.3. Kinetics and Metabolism

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

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

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

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

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

3.1.4. Acute Toxicity

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

3.1.5. Subchronic or Chronic Systemic Toxic Effects

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

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

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

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

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

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

3.1.6. Effects on Nervous System

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

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

3.1.7. Effects on Immune System

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

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

Nonetheless, the toxicity of tebufenozide has been examined in numerous acute, subchronic, and chronic bioassays. Although many of these studies did not focus on the immune system, changes in the immune system (which could potentially be manifest as increased susceptibility to infection compared to controls) were not observed in any of the available long-term animal studies (Appendix 2). Typical subchronic or chronic animal bioassays conduct morphological assessments of the major lymphoid tissues, including bone marrow, major lymph nodes, spleen and thymus (thymus weight is usually measured as well), and blood leukocyte counts. These assessments can detect signs of inflammation or injury indicative of a direct toxic effect of the chemical on the lymphoid tissue. Changes in cellularity of lymphoid tissue and blood, indicative of a possible immune system stimulation or suppression, can also be detected (Durkin and Diamond 2002). None of these effects have been noted in any of the longer term toxicity studies on tebufenozide (Appendix 2).

3.1.8. Effects on Endocrine System

The *endocrine system* participates in the control of metabolism and body composition, growth and development, reproduction, and many of the numerous physiological adjustments needed to maintain constancy of the internal environment (*homeostasis*). The *endocrine system* consists of *endocrine glands*, *hormones*, and *hormone receptors*. *Endocrine glands* are specialized tissues that produce and export (*secrete*) *hormones* to the bloodstream and other tissues. The major endocrine glands in the body include the adrenal, hypothalamus, pancreas, parathyroid, pituitary, thyroid, ovary, and testis. Hormones are also produced in the gastrointestinal tract, kidney, liver, and placenta. *Hormones* are chemicals produced in endocrine glands that bind to *hormone receptors* in target tissues. Binding of a hormone to its receptor results in a process known as *postreceptor activation* which gives rise to a *hormone response* in the target tissue, usually an adjustment in metabolism or growth of the target tissue. Examples include the release of the hormone *testosterone* from the male testis, or *estrogen* from the female ovary, which act on receptors in various tissues to stimulate growth of sexual organs and development of male and female sexual characteristics. The target of a hormone can also be an endocrine gland, in which case, receptor binding may stimulate or inhibit hormone production and secretion. Adverse effects on the endocrine system can result in abnormalities in growth and development, reproduction, body composition, homeostasis (the ability to tolerate various types of stress), and behavior.

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

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

3.1.9. Reproductive and Teratogenic Effects

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

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

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

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

3.1.10. Carcinogenicity and Mutagenicity

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

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

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

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

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

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

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

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

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

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

3.1.13. Inhalation Exposure

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

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

3.1.14. Inerts and Adjuvants

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

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

3.1.15. Impurities and Metabolites

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

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

3.1.16. Toxicologic Interactions

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

For this risk assessment, two exposure scenarios are developed for each of the two types of dermal exposure, and the estimated absorbed dose for each scenario is expressed in units of mg chemical/kg body weight. Both sets of exposure scenarios are summarize in Worksheet E01, which references other worksheets in which the specific calculations are detailed.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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– i.e., annual rainfall of 10 inches of less. At higher rainfall rates, the modeled peak concentrations in streams range from about 0.04 µg/L (loam at an annual rainfall rate of 15 inches) to about 40 µg/L (clay soil at an annual rainfall rate of 150 inches per year) (Table 3-2). While not detailed in Table 3-2, the losses from clay are about equally divided between sediment loss (about 51%) and runoff loss (about 49%). Water contamination due to percolation is negligible (a proportion of about 8×10^{-9}). In sandy soils, however, percolation accounts for virtually all of the total loss at an annual rainfall rate of 250 inches.

Modeled concentrations in a small pond (Table 3-3) are lower than those modeled in the stream. As with the stream modeling, no surface water contamination is expected in very arid regions. For regions with annual rainfall rates of 15 inches or more, the modeled peak concentrations in ponds range from less than 0.006 µg/L (loam) to about 20 µg/L (clay soil at an annual rainfall rate of 250 inches per year). The GLEAMS scenarios do not specifically consider the effects of accidental direct spray. As discussed above and detailed in Worksheet A04b, direct spray of a standard pond could result in peak concentrations of about 6.7 µg/L, somewhat less than the 20 µg/L peak concentration modeled in ponds.

3.2.3.4.4. Other Modeling Efforts – A summary of the GLEAMS modeling discussed above as well as modeling of tebufenozide conducted for other analyses is given in Table 3-4. In addition to GLEAMS, two other water contamination models were used: GENEEC and Sci-Grow. As discussed in SERA (2004b), these are Tier 1 screening models developed by the U.S. EPA that are intended to provide very conservative upper range estimates of concentrations of a compound in surface water (GENEEC) and groundwater (Sci-Grow) based on a given application rate, number of applications, the interval between applications, and standard environmental fate parameters for a specific compound (i.e., a subset of those summarized in Table 3-1).

Estimates of peak concentrations from GENEEC, about 8µg/L, are similar to the central estimates from GLEAMS, 5 to 10 µg/L, but are somewhat less than the peak estimates from GLEAMS, 20 to 40 µg/L. This suggests that although GENEEC is designed as a very conservative model, the application of GLEAMS to the modeling for tebufenozide incorporated more extreme scenarios for contamination. As detailed in SERA (2004b), the application of GLEAMS is intended to encompass extreme situations which favor high runoff from clay and high percolation losses from sand. GENEEC does not provide direct estimates of annual average concentration but does provide 90-day average concentrations. Adjusting the GENEEC modeled 90-day average of 6 µg/L over a one-year period, the concentration of 1.5 µg/L is very close to the upper range of the average concentration modeled using GLEAMS – i.e., 1.4 µg/L for the pond. Sci-Grow estimates a ground water concentration of about 0.09 µg/L. This is in the lower range of the estimates from GLEAMS.   This is probably due to the very shallow root zone used in the GLEAMS modeling – i.e., 12 inches – compared to the 8 to 25 feet water table depth used in Sci-Grow (http://www.epa.gov/oppefed1/models/water/scigrow_description.htm#characteristics).

The only other modeling effort encountered for tebufenozide is the use of PRZM/EXAMS by the U.S. EPA (1999e) for the reregistration of tebufenozide. As summarized in Table 3-4, the U.S. EPA (1999e) modeled the application of tebufenozide to an apple orchard (6 applications at 0.31 lb/acre) and to a cotton field (4 applications at 0.25 lb/acre) for a pond. While this modeling effort used assumptions and weather data substantially different from the GLEAMS modeling (i.e., application rates, soil types, and rainfall patterns), the results are reasonably consistent with the above estimates of concentrations in surface waters based on GLEAMS correcting for differences in the total amount of tebufenozide applied. In the modeling of applications to cotton at a cumulative application rate of 1 lb/acre, for example, the peak concentration estimated by U.S. EPA (1999e) is 17 µg/L. The GLEAMS model was run at a cumulative application of 0.24 lb/acre and the adjusted peak concentration for a pond from U.S. EPA (1999e) would be about 4 µg/L [17 µg/L × 0.24 = 4.08 µg/L], very close to the central estimate of 5 µg/L modeled using GLEAMS. The average annual concentration modeled by U.S. EPA (1999e) was about 8.2 µg/L, which would correspond to 2 µg/L [8.2 µg/L × 0.24 = 1.96 µg/L] at an application rate of 0.24 lb/acre. This is only modestly higher than the peak concentration from GLEAMS of 1.4 µg/L.

　　　　3.2.3.4.5. Monitoring Data – Very little water monitoring data are available on tebufenozide. Although the USGS (1998) provides information on the agricultural uses of tebufenozide, no monitoring data on tebufenozide are available from the USGS National Water Quality Assessment (NAWQA). Sundaram et al. (1996a) published a monitoring study of concentrations of tebufenozide in water that might be associated with the application of this pesticide in a forest environment. In this study, tebufenozide was aerially applied at a rate of 70 g/ha (0.07 kg/ha or 0.06244 lb/acre) to a 500 ha boreal forest. Two applications were made at 4 days apart. Water concentrations were then monitored in a small pond and stream. The pond had a surface area of 500 m^2 and an average depth of 0.6 m for a volume of 300 m^3 or 300,000 L [1,000 L/m^3]. Water concentrations were monitored at 1, 8, and 12 hours after application as well as 1, 2, 3, 4, 5, 8, 12, and 24 days after application.

The peak concentration, 5.31 ppb (0.00531 mg/L) occurred 1 hour after the first application, clearly indicating that the water had been directly sprayed. Taking the water volume of 300,000 L, the amount applied to the pond can be calculated as, 1,593 mg,

$$0.00531 \text{ mg/L} \times 300,000 \text{ L.}$$

The nominal application rate of 0.07 kg/ha is equivalent to 70,000 mg/10,000 m^2 or 7 mg/m^2. At this nominal application rate, the total amount applied to a 500 m^2 pond would be 3500 mg,

$$7 \text{ mg/m}^2 \times 500 \text{ m}^2.$$

Thus, it appears that the initial concentrations of tebufenozide in water are consistent with the direct spray of about 50% [1,593 mg/3500 mg = 0.455 ≈ 50%] of the pond at the nominal application rate.

3.2.3.4.6. Concentrations of Tebufenozide in Water Used for Risk Assessment – A summary of the concentrations of tebufenozide in water that are used for the current risk assessment is given in Table 3-5. The upper range of the expected peak concentration of tebufenozide in surface water will be taken as 40 µg/L. This is based on the upper range of concentrations estimated in streams from the GLEAMS modeling. This concentration also encompasses accidental direct sprays of both a small stream and small pond (Table 3-4). In most instances, concentrations in surface water are likely to be much lower. At the lower extreme, an argument may be made that concentrations of tebufenozide are likely to be essentially zero – i.e., applications at sites that are distant from open bodies of water and in areas in which runoff or percolation are not likely to occur. For this risk assessment, the lower range of the peak concentration in ambient water will be set at 0.005 µg/L. This is in the lower range of non-zero concentrations modeled in streams and ponds in relatively arid regions. The central estimate of concentration of tebufenozide in surface water will be taken as 10 µg/L. This is the central estimate of the concentrations modeled in ponds (Table 3-4).

Longer term concentrations of tebufenozide in surface water will be much lower than peak concentrations. At an application rate of 0.12 lb/acre, the highest longer term concentration will be taken as 1.4 µg/L. This is the maximum longer term concentration modeled using GLEAMS and is near the maximum longer term concentration given by U.S. EPA (1999e) after adjusting for differences in application rate. As with peak concentrations, the lower range of longer term concentrations will approach zero. For this risk assessment, the lower range of longer term concentrations is taken as 0.002 µg/L, the lowest non-zero value modeled for tebufenozide in ponds at the application rate of 0.12 lb/acre. This lower range is somewhat arbitrary but has no impact on the risk assessment. The central value for longer term concentrations of tebufenozide in water will be taken as 0.5 µg/L. This is the central estimate of the longer term concentrations in ponds modeled using GLEAMS and is somewhat higher than the central estimate of the longer term concentration in streams (Table 3-4).

3.2.3.5. Oral Exposure from Contaminated Fish – Many chemicals may be concentrated or partitioned from water into the tissues of animals or plants in the water. This process is referred to as bioconcentration. Generally, bioconcentration is measured as the ratio of the concentration in the organism to the concentration in the water. For example, if the concentration in the organism is 5 mg/kg and the concentration in the water is 1 mg/L, the bioconcentration factor (BCF) is 5 L/kg [5 mg/kg ÷ 1 mg/L]. As with most absorption processes, bioconcentration depends initially on the duration of exposure but eventually reaches steady state. Details regarding the relationship of bioconcentration factor to standard pharmacokinetic principles are provided in Calabrese and Baldwin (1993).

The bioconcentration of tebufenozide was determined in fathead minnows (Rhodes and Leak 1996) and bluegill sunfish (Dong and Hawkins, 1993). In fathead minnows, bioconcentration factors (BCF) range from about 17 in pre-spawn adults to greater than 100 in newly fertilized embryos (Rhodes and Leak 1996). In bluegills, Dong and Hawkins (1993) provide data on bioconcentration in the edible muscle (BCF=7.5) as well as viscera (BCF=106) and whole body

(BCF=52). For the human health risk assessment, the bioconcentration factor of 7.5 from Dong and Hawkins (1993) is used. Taking the value for the edible portion of fish is not the most conservative approach but seems the most realistic approach because humans usually clean caught fish and consume only the fillet or muscle. For the ecological risk assessment, however, the higher BCF value of 52 (whole body) is used.

For the acute and longer-term exposure scenarios involving the consumption of contaminated fish, the water concentrations of tebufenozide used are identical to the concentrations used in the contaminated water scenarios (Section 3.2.3.4.6). The acute exposure scenario is based on the assumption that an adult angler consumes fish taken from contaminated water shortly after an accidental spill of 200 gallons of a field solution into a pond that has an average depth of 1 m and a surface area of 1000 m^2 or about one-quarter acre. No dissipation or degradation is considered.

Bioconcentration is a dynamic process and for some compounds time to maximum steady state may be prolonged. For tebufenozide, Dong and Hawkins (1993) found that time to steady state was reached in about 1-day. Thus, the use of the experimental BCF for the acute accidental scenario is not overly conservative. Nonetheless, this scenario may somewhat overestimate exposure in that some degradation of tebufenozide could occur during the course of the acute spill scenario.

Because of the available and well-documented information and substantial differences in the amount of caught fish consumed by the general public and native American subsistence populations (U.S. EPA 1996), separate exposure estimates are made for these two groups, as illustrated in Worksheet D08a and D08b. The chronic exposure scenario is constructed in a similar way, as detailed in Worksheets D09a and D09b.

3.2.3.6. Oral Exposure from Contaminated Vegetation – Although Forest Service applications of tebufenozide will not involve the intentional treatment of food crops, incidental exposure to vegetation that may be consumed by members of the general public is plausible during broadcast applications. Any number of scenarios could be developed involving either accidental spraying of crops or the spraying of edible wild vegetation, like berries. The exposure scenarios developed for this exposure assessment include one scenario for acute exposure, as defined in Worksheet D03 and two scenarios for longer-term exposure, as defined in Worksheets D04a and D04b. In both acute and longer-term scenarios, the concentration of tebufenozide on contaminated vegetation is estimated using the empirical relationships between application rate and concentration on vegetation developed by Fletcher et al. (1994) which is in turn based on a re-analysis of data from Hoerger and Kenaga (1972). These relationships are defined in Worksheet B20.

For the acute exposure scenario involving only a single application (Worksheet D03a), the estimated residue level is taken as the product of the application rate and the residue rate for contaminated fruit. For multiple applications, the peak concentration on fruit or other vegetation will occur immediately after the last application. This concentration can be calculated based on

the initial concentration after the first application (C_0), the number of applications (n), and the first-order decay coefficient (k), which can be calculated from the halftime (t_{50}) [$k=\ln(2) \div t_{50}$]. Assuming a first-order decrease in concentrations in contaminated vegetation, the concentration in the vegetation at time t after the first application (C_t), can be calculated as:

$$C_t = C_0 \times e^{-kt} \qquad \qquad \text{(Eq. 3-1)}$$

Using the plateau principle (e.g., Goldstein et al. 1974, p. 321) and defining Δt as the interval between applications and $e^{-k \Delta t}$ as p to simplify notation, the concentration immediately after the nth application (C_n) can be calculated as:

$$C_n = C_0 \times (1 - p^n) \div (1 - p). \qquad \qquad \text{(Eq. 3-2)}$$

This algorithm is used in Worksheet D03b to calculate the maximum concentration on vegetation after multiple applications at the specified interval.

For the longer-term exposure scenario (Worksheets D04a and D04b), a duration of 90 days is used. Although the duration of exposure of 90 days is somewhat arbitrarily, this duration is intended to represent the consumption of contaminated fruit that might be available over one season. Longer durations could be used for certain kinds of vegetation but would lower the estimated dose (i.e., would reduce the estimate of risk).

The reported halftimes on vegetation are highly variable (Table 2-1), ranging from 2.8 days, the lower value of the range reported by Hawkins (1998) to 58.7 days, the upper value of the range reported by Sundaram et al. (1996a). This substantial variability is not uncommon in field measurements of halftimes of vegetation, which are substantially impacted by site and situational differences such as rainfall, temperature, wind velocity, and the type of vegetation. For this risk assessment, the range of vegetation halftimes will be taken as 3 to 60 days (the approximate range summarized in Table 2-1) and the central estimate will be taken as 13.4 days, the geometric mean of this range.

For the longer-term exposure scenarios, the time-weighted average concentration on fruit is calculated from the equation for first-order dissipation. Assuming a first-order decrease in concentrations in contaminated vegetation, the concentration in the vegetation at time t after spray, C_t, can be calculated based on the initial concentration, C_0, as:

$$C_t = C_0 \times e^{-kt}$$

where k is the first-order decay coefficient which can be calculated from the halftime (t_{50}) [$k=\ln(2) \div t_{50}$]. For a single application, the time-weighted average concentration (C_{TWA}) over time t can be calculated as the integral of C_t (De Sapio 1976, p. p. 97 ff) divided by the duration (t):

$$C_{TWA} = C_0 (1 - e^{-k\,t}) \div (k\,t).$$

This equation is used to estimate the time-weighted average concentration on vegetation after a single applications (Worksheet D04a).

For two applications, such as those modeled in this risk assessment, the expression of the time-weighted average concentration is somewhat more complicated. Defining *exp(x)* as e^x, where *x* is any number, the time-weighted average concentration over a period from the day of application to time t_2 with a second application occurring on day t_1 (where $t_1 \leq t_2$) is:

$$C_{TWA} = (C_0 (1\text{-}exp(\text{-}kt_1)) + [\{C_0 + C_0\, exp(\text{-}kt_1)\} \times \{1\text{-}exp(\text{-}k\, [t_2 \text{-} t_1])\}]) \div (k\, t_2)$$

This equation is used to estimate the time-weighted average concentration on vegetation after a single applications (Worksheet D04b).

3.3. DOSE-RESPONSE ASSESSMENT
3.3.1. Overview
Acute and chronic risk values are derived for tebufenozide. Following standard practices for USDA risk assessments, risk assessment values available from U.S. EPA are adopted directly unless there is a compelling basis for doing otherwise. When risk values are not available from U.S. EPA, the methods used by U.S. EPA are employed to derive surrogate values.

U.S. EPA has derived a chronic RfD for tebufenozide of 0.018 mg/kg/day. This chronic RfD is well-documented and is used directly for all longer term exposures to tebufenozide. This value is based on a NOAEL of 1.8 mg/kg/day in dogs and an uncertainty factor of 100 – two factors of 10 for interspecies and intraspecies variability. Because of the low acute toxicity of tebufenozide, the U.S. EPA has not derived an acute RfD but has identified an acute NOAEL of 1000 mg/kg/day from reproduction studies in both rats and rabbits involving 10 to 13 day exposure periods. This NOAEL is the basis for a surrogate acute RfD of 10 mg/kg using an uncertainty factor of 100 as in the chronic RfD. This surrogate acute RfD is applied to all incidental or accidental exposures that involve an exposure period of 1 day.

3.3.2. Chronic RfD
The most recent RfD for tebufenozide is 0.018 mg/kg/day, a value derived by the U.S. EPA's Office of Pesticide Programs (U.S. EPA 1999b,e). This compound is not listed on the U.S. EPA's agency-wide list of approved RfDs (i.e., IRIS) (U.S. EPA 2004). As noted in section 3.1.2 and detailed in Appendix 2, the most sensitive endpoint for tebufenozide is hematological effects including methemoglobin formation and several other endpoints that are characteristic of hemolytic anemia. These effects were observed in mice, rats, and dogs, with the dog being the most sensitive species tested with tebufenozide. As reviewed by Calabrese (1991), this pattern is consistent with known differences in methemoglobin reductase activity which suggest that the cat may be the most sensitive species, followed by humans (half as susceptible as cats), dogs (half as susceptible as human), and rats (about one-tenth as susceptible as humans).

The RfD derived by the U.S. EPA (1999b) is based on a study by Richards (1992a,b) in which a dietary concentration of 0, 15, 50, 250, or 1500 ppm technical grade tebufenozide was provided to male and female beagles for 52 weeks (Appendix 2). In the 250 and 1500 ppm groups, the primary hematological effects were increased concentrations of methemoglobin. The increases in methemoglobin concentrations were associated with increased breakdown of red blood cells in the liver and spleen, and decreases in red blood cell counts, hemoglobin concentrations, and packed red cell volume, along with several other associated hematological effects. None of these effects were observed in beagles exposed to a dietary concentration of 50 ppm technical grade tebufenozide, which corresponded to a daily dose of 1.5-2.4 mg/kg bw (based on measured food consumption). Taking 1.8 mg/kg bw/day as a central estimate of the NOAEL, the U.S. EPA (1999b) applied an uncertainty factor of 100, two factors of 10 for interspecies and intraspecies variability, to arrive at the chronic RfD of 0.018 mg/kg/day.

Under the Food Quality Protection Act (FQPA), the U.S. EPA is required to consider an

additional uncertainty factor of 10 for the protection of infants and children. For tebufenozide, the U.S. EPA (1999b) determined that the additional uncertainty factor is not required because of the information indicating that tebufenozide does not have developmental or reproductive effects at doses below those associated with hematological effects. Hence, because the RfD should protect against hematological effects, it should also protect against developmental or reproductive effects. As discussed in Section 3.4.4, infants less than three months old have lower levels of methemoglobin reductase than older children or adults and may be more sensitive tebufenozide and other agents that cause methemoglobinemia. While it may be argued that an uncertainty factor for very young children might be appropriate, this would not have an impact on the risk characterization because of the very low hazard quotients associated with various exposure scenarios for tebufenozide (Section 3.4.3).

3.3.3. Acute RfD

The U.S. EPA (1999b) considers the acute and intermediate risk from acute or intermediate exposure to tebufenozide negligible and does not propose short-term or intermediate-term criteria for exposure to tebufenozide. Specifically, the U.S. EPA (1999b) made the following judgement:

> *1. Acute toxicity. Toxicity observed in oral toxicity studies were not attributable to a single dose (exposure). No neuro or systemic toxicity was observed in rats given a single oral administration of tebufenozide at 0, 500, 1,000, or 2,000 mg/kg. No maternal or developmental toxicity was observed following oral administration of tebufenozide at 1,000 mg/kg/day (Limit-Dose) during gestation to pregnant rats or rabbits. Thus, the risk from acute exposure is considered negligible.*
>
> *2. Short- and intermediate-term toxicity. No dermal or systemic toxicity was seen in rats receiving 15 repeated dermal applications of the technical (97.2%) product at 1,000 mg/kg/day (Limit-Dose) as well as a formulated (23% a.i.) product at 0, 62.5, 250, or 1,000 mg/kg/day over a 21-day period. The Agency noted that in spite of the hematological effects seen in the dog study, similar effects were not seen in the rats receiving the compound via the dermal route indicating poor dermal absorption. Also, no developmental endpoints of concern were evident due to the lack of developmental toxicity in either rat or rabbit studies. This risk is considered to be negligible. -- U.S. EPA (1999b).*

In paragraph 1 above, the acute toxicity study with a single-dose NOAEL of 2000 mg/kg appears to refer to the study by Swenson et al. (1994) and the NOAEL of 1000 mg/kg/day for maternal toxicity and reproductive effects in rats and rabbits appears to refer to the studies by Hoberman (1991) and Swenson and Solomon (1992), respectively. In paragraph 2 above, the U.S. EPA (1999b) refers to a dermal study with a NOAEL of 1000 mg/kg/day. In this study, tebufenozide

was applied 5 days per week for three weeks – i.e., 15 exposures over a 21 day period.   Two repeated dermal dose studies have been identified with a NOAEL of 1000 mg/kg/day (Hazleton and Quinn 1995b; Morrison et al. 1993). As summarized in Appendix 3, both of these studies report exposure periods of 4 weeks rather than 3 weeks.

While the decision of the U.S. EPA (1999b) to classify acute and short-term risks associated with tebufenozide appears reasonable, the failure of the U.S. EPA (1999b) to derive an acute RfD limits the ability to quantitatively characterize risks associated with acute exposures. As detailed in Section 3.2, the current risk assessment is concerned with characterizing the risks of several acute exposure scenarios. In addition, the current risk assessment is part of a series of risk assessments on different agents used to control the gypsy moth the estimates of risks from the various agents will be compared in a companion document.

Consequently, this risk assessment will use a surrogate acute RfD. Typically, the U.S. EPA will base acute RfDs on reproduction studies, specifically teratology studies that involve multiple daily gavage doses to pregnant animals. For the current risk assessment, the NOAEL of 1000 mg/kg/day in pregnant rats and rabbits identified by U.S. EPA (1999b) will be used   As detailed in Appendix 2, the NOAEL in rabbits is from a study (Swenson and Solomon 1992) in which animals were dosed on Days 7-19 of gestation – i.e., repeated exposures over 13 days – and the NOAEL in rats is from a study (Hoberman 1991) in which animals were dosed on Days 6-15 of gestation – i.e., repeated exposures over 10 days. Dividing this NOAEL by an uncertainty factor of 100, identical to that used by U.S. EPA (1999b) in the chronic RfD, yields a surrogate acute RfD of 10 mg/kg/day. This value is used to characterize risks associated to incidents or accidents that involve an exposure period of 1 day.

3.4. RISK CHARACTERIZATION
3.4.1. Overview
At the maximum application rate considered in this risk assessment, two applications at 0.12 lb/acre spaced three day apart, there is little indication that adverse effects on human health are likely. Based on central estimates of exposure – those that might be considered typical and expected – hazard quotients including workers and members of the general public range from 0.00003 to 0.03, below a level of concern by factors of about 30 to 33,000. At the upper range of plausible exposures, the hazard quotient for ground spray workers reaches a level of concern – i.e., a hazard quotient of 1. For members of the general public, the upper range of exposure leads to a hazard quotient of 1.5 for the longer-term consumption of contaminated vegetation for two applications at 0.12 lb/acre. Because of the linear relationship between exposure and application rate, two applications at 0.08 lb/acre would reach but not exceed a level of concern. With a single application at the maximum rate of 0.12 lb/acre, the hazard index is 0.8, below the level of concern. While the longer-term consumption of contaminated vegetation is probably not a likely scenario, it is a standard exposure scenario used in Forest Service risk assessments to consider the longer-term consumption of food items such as berries that might be sprayed during the broadcast application of a pesticide. This risk assessment suggests that two applications at 0.08 lb/acre or more should be avoided in areas where members of the general public might consume contaminated fruits or other contaminated vegetation.

3.4.2. Workers
A quantitative summary of the risk characterization for workers is presented in Worksheet E02 (Supplement 1). The quantitative risk characterization is expressed as the hazard quotient, which is the ratio of the estimated exposure from Worksheet E01 to the RfD. For acute accidental/incidental exposures, the surrogate acute RfD of 10 mg/kg is used (Section 3.3.3). For longer term general exposures – i.e., exposures that could occur over the course of several days, weeks, or months during an application season – the chronic RfD of 0.018 mg/kg/day is used (Section 3.3.2).

At the maximum application rate considered in this risk assessment, 0.12 lb/acre, none of the acute hazard quotients exceed a level of concern – i.e., a hazard quotient of 1. The highest acute hazard quotient is 0.4, associated with wearing contaminated gloves for 1 hour. It should be noted, however, that the magnitude of the hazard quotient is linearly related to the duration of exposure. The 1-hour exposure period is simply a convention that is uniformly used in Forest Service risk assessments (SERA 2001). For tebufenozide, the estimated exposure would exceed the acute RfD – i.e., result in a hazard quotient greater than 1 – if a worker were to wear contaminated gloves for a period greater than 2.5 hours. Thus, the exposure involving contaminated gloves is of greatest concern and this concern would apply to wearing any clothing that is saturated with tebufenozide.

For longer-term exposures, the highest hazard quotient is 1.008 and is associated with the upper range of exposure for ground spray workers at the maximum application rate of 0.12 lb/acre. In Worksheet E02, this value is presented as 1.0 – i.e., rounded to one significant place after the

decimal. This very minor exceedence of the chronic RfD is interpreted as a hazard quotient of 1.0 – i.e., the level of concern is not exceeded. All of the other hazard quotients are below a level of concern by a factor of at least 2 at the upper range of exposures and a factor of at least 10 at the central estimates of exposure. It should be noted that multiple applications of tebufenozide, such as those covered in this risk assessment, have no effect on the hazard quotients for workers. This is because all worker exposure assessments are based on the assumption that the worker applies the compound daily, albeit at different sites, over the course of an application season.

Mimic can cause eye irritation (section 3.1.11). Quantitative risk assessments for irritation are not derived; however, from a practical perspective, eye irritation is likely to be the only overt effect as a consequence of mishandling tebufenozide. This effect can be minimized or avoided by prudent industrial hygiene practices during the handling of the compound.

3.4.3. General Public

A quantitative summary of the risk characterization for members of the general public is presented in Worksheet E04 (Supplement 1). With the exception of the scenarios for the longer-term consumption of contaminated vegetation, all exposure scenarios are based on the highest application considered in this risk assessment – i.e., two applications at a rate of 0.12 lb/acre with an interval of 3 days between applications. Two scenarios are conducted for the longer-term consumption of contaminated vegetation, one involving two applications spaced three days apart and the other involving only a single application. Both are modeled at the maximum rate of 0.12 lb/acre. As with the risk characterization for workers, risk is expressed quantitatively as the hazard quotient using the surrogate acute RfD of 10 mg/kg (Section 3.3.3) for acute exposures and the chronic RfD of 0.018 mg/kg/day (Section 3.3.2) for longer-term exposures.

The only exposure scenario that leads to any unacceptable risk is the longer-tern consumption of contaminated vegetation. For two applications spaced three days apart at the maximum rate of 0.12 lb/acre, the hazard quotient 1.5 for the longer-term consumption of contaminated vegetation – i.e., the exposure exceeds the RfD by a factor of 1.5. Because the exposure is linearly related to the application rate, two exposures at an application rate of 0.08 lb/acre [0.12 lb/acre ÷ 1.5] would reach but not exceed the level of concern. With a single application at the maximum rate of 0.12 lb/acre, the hazard index is 0.8, below the level of concern. As discussed in Section 3.2.3.6, this exposure scenario assumes that an individual will consume over a 90 day period after that fruit had been directly sprayed. The probability of this occurring is unlikely because the USDA will not intentionally apply tebufenozide to crops or other food items. Nonetheless, this is a standard exposure scenario used in Forest Service risk assessments to consider the longer-term consumption of food items such as berries that might be sprayed during the broadcast application of a pesticide. This risk assessment suggests that two applications at 0.08 lb/acre or more should be avoided in areas where members of the general public might consume contaminated fruits.

None of the acute or other longer-term hazard quotients exceed 1 even at the upper ranges of

plausible exposure. The highest acute hazard quotient is 0.1, the upper range of risk for the consumption of contaminated water by child after an accidental spill. This extreme and accidental acute scenario is below the level of concern by a factor of 10. No other acute exposure scenarios, many of which involve extremely conservative assumptions, approach a level of concern at the upper range of exposure. Based on central estimates of exposure, which involve somewhat less conservative assumptions, the acute hazard quotients range from 0.00008 to 0.02 – i.e., below the level of concern by factors of 50 to 12,500. Based on central estimates of longer-term exposures, the hazard quotients range from 0.00003 to 0.03, below the level of concern by factors of about 30 to over 33,000.

3.4.4. Sensitive Subgroups

Some individuals are born with a form of congenital methemoglobinemia and may be at increased risk of adverse effects to compounds that induce methemoglobinemia (Centa et al. 1985; Das Gupta et al. 1980). Infants less than 3 months old have lower levels of methemoglobin (cytochrome b5) reductase and higher levels of methemoglobin (1.32%), compared with older children or adults (Centa et al. 1985; Smith 1996). A similar pattern is seen in many species of mammals (Lo and Agar 1986). Thus, it is possible that infants could be more sensitive to the effects of tebufenozide than adults.

3.4.5. Connected Actions

The most sensitive effect for tebufenozide, methemoglobinemia, is also associated with exposures to diflubenzuron, another agent used for gypsy moth control. These two agents are likely to have an additive effect on methemoglobinemia but these agents are not used together. Thus, simultaneous exposures are unlikely. Exposure to other compounds in the environment that induce methemoglobinemia may also lead to an additive effect. Any agent or condition that may reduce the oxygen carrying capacity of the blood could lead to increased risks from exposure to either tebufenozide or diflubenzuron. For example, individuals exposed to combustion smoke or carbon monoxide (that is, agents that do oxidative damage to blood) may be at increased risk of developing methemoglobinemia (Hoffman and Sauter 1989; Laney and Hoffman 1992). In addition, individuals exposed to high levels of nitrates, either in air or in water, will have increased levels of methemoglobin (Woebkenberg et al. 1981) and may be at increased risks of exposure to compounds such as tebufenozide.

3.4.6. Cumulative Effects

This risk assessment is based on two applications at the maximum allowable rate of 0.12 lb/acre. This approach is used to estimate maximum daily exposure and daily absorbed dose. In addition, this risk assessment specifically considers the effect of repeated exposure in that the chronic RfD is used as an index of acceptable longer-term exposures and an acute RfD based on an exposure period of 10 to 13 days is used for the risk characterization of single day exposures. Consequently, the risk characterizations presented in this risk assessment specifically addresses and encompasses the potential impact of long-term exposure and cumulative effects.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview. The toxicity of tebufenozide is well characterized in experimental mammals, birds, terrestrial invertebrates, and aquatic animals. Nonetheless, given the very large number of species in the environment which could be exposed to tebufenozide, toxicity data are available on relatively few species.

It seems reasonable to assume the most sensitive effects in wildlife mammalian species will be the same as those in experimental mammals (i.e., effects on the blood, specifically the formation of methemoglobin, which leads to a spectrum of other effects in blood that can be characterized as hemolytic anemia). At higher doses, tebufenozide was associated with impaired reproductive performance in experimental mammals, and this effect is also considered quantitatively in this risk assessment. Potential reproductive effects are also of concern for birds, although there are inconsistencies in the available experimental data. The available literature includes a reproduction study investigating effects in mallard ducks and two reproduction studies investigating effects in bobwhite quail. In one of the quail studies, dietary concentrations of 300 and 1000 ppm caused reproductive effects. These effects were not observed in that study at 100 ppm or in the more recent quail study or in the study on mallard ducks. A field study on the effects of tebufenozide on reproductive performance in birds noted trends that were statistically insignificant but suggestive of adverse reproductive effects in a warbler species. Thus, consistent with the interpretation by the U.S. EPA, reproductive effects in both mammals and birds are considered endpoints of concern in this risk assessment.

The mechanism of action of tebufenozide in target insects is relatively well understood. Tebufenozide mimics the action of the invertebrate hormone, 20-hydroxyecdysone, which controls molting. The effectiveness of tebufenozide in mimicking 20-hydroxyecdysone activity, however, appears to vary markedly among orders and species of invertebrates. In general, lepidopteran species are sensitive to tebufenozide but other insects are much less sensitive.

There are no bioassays regarding the toxicity of tebufenozide to terrestrial plants or terrestrial microorganisms in the literature. There are a number of field studies and field simulation studies available on tebufenozide and effects that might be associated with toxicity to plants or soil microorganisms have not been noted.

The acute toxicity of tebufenozide to aquatic animals is relatively low, with acute LC_{50} values ranging from 2.2 to 6.5 mg/L for fish and 0.3 to 3.8 mg/L for aquatic invertebrates. Nonetheless, much lower concentrations of tebufenozide may cause reproductive effects in fish (0.048 mg/L) and aquatic invertebrates (0.0053 mg/L).

4.1.2.   Toxicity to Terrestrial Organisms.
4.1.2.1. Mammals– As summarized in the human health risk assessment (see Section 3.1), the mode of action of tebufenozide in mammals is relatively well characterized. Several standard toxicity studies in experimental mammals were conducted as part of the registration process (Appendix 2).   The most sensitive effect in several species of experimental mammals involves effects on the blood, specifically the formation of methemoglobin, which leads to a spectrum of other effects in blood that can be characterized as hemolytic anemia. Since higher doses of tebufenozide were associated with impaired reproductive performance (see Section 3.1.4), both toxic and reproductive effects are considered in this risk assessment.

The acute toxicity of tebufenozide is relatively low, with an oral LD50 greater than 5000 mg/kg. The subchronic and chronic toxicity studies on tebufenozide were conducted in dogs, mice, and rats. The most sensitive effects involve changes to blood. The most sensitive species is the dog, with a NOAEL of 50 ppm in the diet (1.8 mg/kg bw/day) and an effect level of 500 ppm (about 20 mg/kg bw/day) over an exposure period of 1 year.

As discussed in Section 3.3.3, there is no apparent dose duration relationship for tebufenozide. In other words, short-term exposures are likely to lead to changes in the blood comparable to those observed after longer-term exposures. Thus, the chronic NOAEL of 1.8 mg/kg/day is used to characterize risks associated with both short- and long-term exposures.

4.1.2.2. Birds– Toxicity studies have been conducted on the acute toxicity and reproductive effects of tebufenozide in birds and a field study is available on reproductive effects.

Information regarding the laboratory tests on the toxicity of tebufenozide to birds is summarized in Appendix 4. The acute toxicity of tebufenozide is low for birds, as it is for mammals. When administered in gelatin capsules, the 21-day oral LD_{50} is greater than 2150 mg a.i./kg bw (Fletcher 1987). Similarly, in 5-day dietary studies, the dietary LC_{50} is greater than 5000 ppm (Fletcher 1990a,b). Hematological endpoints are not usually assayed in bioassays with birds, and there are no data regarding the hematological effects in birds after exposure to tebufenozide.

Nevertheless, the most relevant and significant studies for this risk assessment involve the potential reproductive effects in birds exposed to tebufenozide. Reproduction studies were conducted in mallard ducks (Beavers et al. 1993a) and bobwhite quail (Beavers et al. 1993b; Reinert 1995a). As indicated in Appendix 4, dietary concentrations less than or equal to 1000 ppm tebufenozide did not cause reproductive effects in mallard ducks In the quail studies, however, the results are inconsistent. In the earlier study by Beavers et al. (1993b), reproductive effects - including a reduced number of eggs laid, viable embryos and 14 day old survivors - were noted at dietary concentrations of 300 and 1000 ppm, but not at 100 ppm. In a similar study conducted later by Reinert (1995a), there were no substantial dose-related effects in quail exposed to dietary concentrations of up to 615 ppm.

In terms of the hazard identification, the most important question involves the extent to which

the Reinert (1995a) study reporting negative results for reproductive toxicity reduces the concerns raised by the Beavers et al. (1993b) study, which reports positive results. The earlier study was accepted by the U.S. EPA (1999e) and used in their ecological risk assessment of tebufenozide; however, the U.S. EPA (1999e) does not discuss the later negative study. The negative study is discussed in a review by Rohm and Haas (Keller and Brown 1998b), who question whether the NOAEL for the earlier study was 100 ppm or 300 ppm.

Regardless of which dose is classified as a NOAEL in the Beavers et al. (1993b) study, there seems to be no evidence that the study is flawed in any way. The minor differences between the early study and the later study, as detailed in Appendix 4, relate primarily to how exposures were reported and how food consumption was measured.

Notably, reproductive effects were observed also in mammals exposed to a dietary concentration of 2000 ppm ($\approx$160 mg/kg bw), with a NOAEL of 150 ppm ($\approx$12 mg/kg bw) (see Section 3.1.4). In the bobwhite quail study conducted by Beavers et al. (1993b), the dietary effect levels (AELs) of 300 and 1000 ppm correspond to estimated daily doses of 45 and 150 mg/kg/day, and the NOAEL of 100 ppm corresponds to an estimated daily dose of 15 mg/kg bw. Thus, the apparent NOAEL values and AEL values for mammals and birds are reasonably consistent. Finally, based on a metabolism study in hens (Sharma and Schuck 1996), the metabolic pathways for birds and mammals appear to be similar.

In the absence of any basis for discounting the earlier study in bobwhite quail (Beavers et al. 1993b) and given the reasonable consistency in dose levels associated with reproductive effects in mammals and birds as well as the similar metabolic pathways in mammals and birds, reproductive effects are considered an endpoint of concern in this risk assessment.

A field study on the reproductive performance of Tennessee warblers (*Vermivora peregrina*) in forests treated with Mimic has been published (Holmes 1998). In this study, Mimic was applied at a rate of 0.07 a.i. kg/ha, approximately 0.06 lb a.i./acre, in a forest area in Ontario. Two applications were made at this rate with a 4 day interval between applications. A number of reproductive parameters were assayed including number of eggs laid, percent hatch and growth of the hatchlings. There parameters were compared to an untreated control plot. A total of six nests were observed in the control plot and 5 nests in the plot treated with Mimic. No statistically significant adverse effects were noted. However, there were decreases in both the average number of eggs per nest (6.3 in the control area and 5.8 in the treated area) as well as the percent hatch (97.4% in the control area and 89.7% in the treated area). As noted by Holmes (1998, p. 191), the small sample sizes result in a low statistical power and the results are *"suggestive, although not necessarily compelling, that reproductive parameters were consistently lower in the treated blocks than in the control block."* Some differences in adult behavior were observed in the plot treated with Mimic – i.e., an increase in foraging time and an associated decrease in brooding time. This suggests that the primary effect on the birds may have been a decrease in food abundance.

This field study by Holmes (1998) combined with bobwhite quail assay conducted by Beavers et al. (1993b) raise concern that tebufenozide could cause adverse reproductive effects in birds. This concern is addressed quantitatively in this risk assessment for exposures involving the consumption of contaminated vegetation, fish, and insects.

4.1.2.3. Terrestrial Invertebrates – While Mimic is specifically used by the Forest Service for the control of the Gypsy moth, tebufenozide is effective in the control of other lepidopteran pest species, including the apple bud moth (*Platynota idaeusalis,* Biddinger et al. 1998), various species of spruce budworm (Cadogan et al. 1997; Payne et al. 1997; Retnakaran et al. 1997a,b), the tomato looper (*Deixis chalcites*, Smagghe et al. 1997), and the Indian-meal moth (*Plodia interpunctella*) (Oberlander et al. 1998). A complete list of the pest species for which tebufenozide is specified is provided in U.S. EPA (1999e).

The toxicity of tebufenozide has been assayed in several species (Appendix 5). The mechanism of action of tebufenozide in target insects is relatively well understood. In sensitive species, tebufenozide mimics the action of the invertebrate hormone 20-hydroxyecdysone. This hormone controls molting in insects and various terrestrial and aquatic invertebrates, which is mediated through binding to species-specific ecdysone receptors present in the cytoplasm of epidermal cells (Addison 1996; Keller 1998; Smagghe and Degheele 1994a; U.S. EPA 1999e).

While 20-hydroxyecdysone is a hormone common to many invertebrates, the effectiveness of tebufenozide in mimicking 20-hydroxyecdysone activity seems to vary markedly among orders and species of invertebrates. Although the specificity of tebufenozide is not addressed in detail in the recent U.S. EPA (1999e) ecological risk assessment, it was reviewed in detail by Rohm and Haas (Keller 1998). The review by Keller (1998) is consistent with publications in the open literature relating to species specificity of tebufenozide (Addison. 1996; Biddinger and Hull. 1995; Biddinger et al. 1998; Brown. 1996; Butler et al. 1997; Dhadialla et al. 1998; Rumpf et al. 1998; Smagghe and Degheele 1994a,b, 1997; Smagghe et al. 1995, 1996a,b; Valentine et al. 1996). In general, *Lepidoptera* are sensitive to tebufenozide but other insects are much less sensitive (Smagghe and Degheele 1994a). The differences in sensitivity appear to be related to differences in ecdysone receptor binding (Smagghe et al. 1996a) rather than differences in pharmacokinetics (Smagghe and Degheele 1994b).

There are four studies regarding the effects of tebufenozide to terrestrial invertebrates under field or field simulation conditions (Appendix 6). Three of these studies are published in the open literature (Addison 1996; Butler et al. 1997; Valentine et al. 1996), and one unpublished study was conducted by Rohm and Haas (Walgenbach 1995). The studies by Addison (1996) and Butler et al. (1997) are most directly relevant to this risk assessment because they assayed the effects on nontarget invertebrates in the forest canopy (Butler et al. 1997) and forest soil (Addison 1996) after the application of tebufenozide.

In the study by Addison (1996), tebufenozide was incorporated into forest soil at a concentration of 72.1 ppm. Based on a typical application rate of 70 g/ha and the assumption that tebufenozide

will remain in the top 2 cm of soil, Addison (1996) estimated that the soil concentration of 72.1 ppm is equivalent to a concentration that is 100 times greater than expected environmental concentrations. There were no adverse effects on one species of earthworm (*Dendrobaena octaedra*) or on four species of Colembola (*Folsomia candida*, *Folsomia nivalis*, *Onychiurus parvicornis*, and *Hypogastrura pannosa*), which are indigenous to forest soils in Canada and the northern United States. Consistent with results of the Addison (1996) study, a standard bioassay on earthworms (*Eisenia foetida*) noted no adverse effects at soil concentrations of up to 1000 ppm over a 14-day exposure period (Garvey 1992).

Butler et al. (1997) conducted a study on canopy arthropods in which Mimic 4F was applied at rates of 0.03 and 0.06 lb a.i./acre to a mixed oak plot in Ohio. The investigators examined Mimic's efficacy against Gypsy moth larvae and its effects on nontarget arthropods. Population assays included measures of abundance and diversity in10 arthropod families and 15 lepidopteran species. No effects on abundance or richness were noted in any organisms other than lepidopteran species. A decrease in abundance was noted in some lepidopteran species.   The study indicates that there were problems associated with the application of Mimic 4F that resulted in poorer than expected efficacy, and that consequently, effects in nontarget lepidopteran species may have been underestimated.

The studies by Valentine et al. (1996) and Walgenbach (1995) involve the application of tebufenozide formulations to apple orchards. The study by Valentine et al. (1996) found no effects of tebufenozide on species of mites, spiders, various beetles (*Coleoptera*), and true bugs (*Hemiptera*) after Mimic was applied to apple orchards at rates that were effective in controlling lepidopteran pest species. Similarly, Walgenbach (1995) noted no effects on beneficial insect populations after Confirm was applied to apple plots. While not as directly relevant to this risk assessment as the forestry studies summarized above, these two studies support the general conclusion that tebufenozide is likely to have an adverse impact on *Lepidoptera* but not on non-lepidopteran species.

In addition to the above studies, the standard bee toxicity assay was conducted on tebufenozide (Atkins. 1990; Chan 1995). In this study, no mortality was observed at doses of up to 233.98 µg a.i./bee. Using a body weight of 0.093 g for the honey bee (USDA/APHIS 1993), this corresponds to a dose of about 2500 mg/kg bw [0.23 mg/0.000093 kg = 2473 mg/kg bw].

***4.1.2.4. Terrestrial Plants (Macrophytes)*–** Standard bioassays for toxicity to terrestrial plants are required by the U.S. EPA for the registration of herbicides but not insecticides. No bioassays for herbicidal activity of tebufenozide were encountered in the published literature or in the U.S. EPA/OPP files. Thus, the potential effects of tebufenozide on terrestrial plant species is not discussed in other reviews of this compound (U.S. EPA 1999d,e; Keller 1998). The implicit presumption is that plausible levels of exposure to tebufenozide will not adversely affect terrestrial plant species.

There are several field studies regarding the efficacy of tebufenozide applied to terrestrial

vegetation for the control of various insect pests (e.g., Biddinger et al. 1998; Cadogan et al. 1997; Oberlander et al. 1998; Payne et al. 1997; Retnakaran et al. 1997a,b; Valentine et al. 1996; West et al. 1997). If tebufenozide were toxic to terrestrial plants at application rates that are used in the field, it is plausible that adverse effects would be reported in this literature. No such reports were encountered.

Because there is no basis for further evaluating the assumption that tebufenozide will not cause adverse effects in terrestrial plants, such effects will not be considered quantitatively in this risk assessment.

4.1.2.5. Terrestrial Microorganisms– As indicated in U.S. EPA (1999e), microbial transformation is the predominant route of environmental degradation in soil and water. Data regarding the toxicity of tebufenozide to terrestrial microorganisms, as with terrestrial plants, is not available in the open literature or the U.S. EPA/OPP files. Tebufenozide is degraded in soil by some microorganisms (e.g., Sundaram 1996, 1997a). Nonetheless, given the diversity of soil microorganisms and soil environments, generalizations concerning the potential effects on soil microflora cannot be supported.

4.1.3. Aquatic Organisms.
4.1.3.1. Fish– Information on the toxicity of tebufenozide to fish is summarized in Appendix 7. All of the available studies were conducted in support of the registration of tebufenozide and submitted to U.S. EPA/OPP. The summaries of these studies given in Appendix 7 were taken from the full text copies of the studies submitted to U.S. EPA.

The acute toxicity of tebufenozide to fish is relatively low – i.e., LC_{50} values of 3.0 mg a.i./L in Bluegill sunfish (Graves and Smith 1992b) and 5.7 mg a.i./L in Rainbow trout (Graves and Smith 1992c). There is greater concern, however, regarding the potential chronic toxicity of tebufenozide to fish. The U.S. EPA evaluates all studies like those summarized in Appendix 7 to determine whether the conclusions from the studies are consistent with the data presented in the studies. In many instances, the U.S. EPA accepts the study conclusions. For tebufenozide, however, the U.S. EPA has disagreed with conclusions for a fathead minnow egg and fry study (Bettancourt 1992) as well as a fathead minnow full life cycle study (Rhodes and Leak 1996). This is discussed further in the dose-response assessment (Section 4.3.3.1).

4.1.3.2. Amphibians– No information was encountered on the toxicity of tebufenozide to amphibians.

4.1.3.3. Aquatic Invertebrates – Unpublished studies on the toxicity of tebufenozide to aquatic invertebrates that were submitted to the U.S. EPA in support of the registration of tebufenozide are summarized in Appendix 8. Some invertebrate assays were conducted in support of the registration of tebufenozide, and the summaries of these studies are based on the full text copies of the studies submitted to U.S. EPA. Additional studies published in the open literature are discussed below. Unlike some of the fish studies, the studies on aquatic invertebrates,

summarized in Appendix 8, were accepted without exception by the U.S. EPA (1999e).

In the studies submitted for registration, the acute toxicity of tebufenozide to daphnia (*Crustacea*) and midges (*Insecta*) is on the same order as that for fish, with a 48 hour LC_{50} value of 3.8 mg/L for daphnids (Graves and Smith 1992a) and a 96 hour LC_{50} value of 0.3 mg/L for midge larvae (van der Kolk 1997). Similarly, in a study published in the open literature and sponsored by the U.S. Geological survey, Song et al. (1997) report higher LC_{50} values for Crustacea (daphnia = 17.37 mg/L; Artemia = 5.53 mg/L) than for two species of mosquitoes (0.92 mg/L for *Aedes aegypti* and 0.15 mg/L for *Aedes taeniorhynchus*). All of these bioassay results from Song et al. (1997) involved exposures at 27°C. In similar bioassays conducted at 20°C, tebufenozide was substantially less toxic to both daphnids and *Aedes aegypti*. This negative relationship between toxicity and temperature is common.

As with fish, there is a concern for potential reproductive effects in both a free swimming species (*Daphnia*) as well as a sediment dwelling species (midge). In *Daphnia magna*, significant decreases in the number of offspring/female were noted at 0.12 mg/L and a significant decrease in the growth of offspring was noted at 0.059 mg/L (McNamara 1991). In midges (*Chironomus riparius*), a decrease in larval emergence was noted at a concentration of 0.0053 mg/L. At concentrations of 0.04 mg/L and higher, midge emergence was completely suppressed (van der Kolk 1997).

Kreutzweiser and Thomas (1995) assayed the effects of tebufenozide on aquatic invertebrate communities in lake enclosures at nominal concentrations of 0.07, 0.13, 0.33, and 0.66 mg/L. A dose-related decrease in cladoceran abundance was noted and persisted for 1-2 months at the two lower concentrations and for 12-13 months at the two higher concentrations. The decrease in cladoceran abundance was accompanied by an increase in the abundance of rotifers, suggesting that the changes in community structure could be attributable to secondary or trophic effects rather than to toxicity.

Rohm and Haas summarized the results of Kreutzweiser and Thomas (1995) along with several other field studies or field simulation studies (e.g. Kreutzweiser et al. 1994) regarding the effects of tebufenozide to aquatic invertebrates (Keller 1998). The most relevant study for this risk assessment is an unpublished report submitted to U.S. EPA (Russell et al. 1996). In this study, Mimic was applied at a rate of 70 g a.i./ha to a small forest pond. The application resulted in an initial concentration of 0.00837 mg/L which decreased to 0.00016 mg/L 1 month after spray. During the 1-month post-application observation period, no adverse effects were noted on invertebrate populations, compared with a control (untreated) pond. Notably, the maximum concentration of 0.00837 mg/L is very close to the effect level of 0.0053 mg/L for midge larvae; however, the average concentration during the 1-month study was probably substantially below the effect level in midges. Thus, although this study seems to support the assertion that tebufenozide can be applied without interfering with aquatic invertebrate communities, it is not in conflict with the available bioassay data.

4.1.3.4. Aquatic Plants – The toxicity of tebufenozide was assayed in two species of freshwater green algae, and details of these studies are presented in Appendix 8 along with the studies on aquatic invertebrates. *Selenastrum capricornutum* appears to be relatively insensitive to tebufenozide, with a NOEC for reduced cell density of 0.64 mg/L (Reinert 1993b), which is greater than the effect levels in aquatic invertebrates by a factor of 10-100.

Scenedesmus subspicatus appears to be much more sensitive than *Selenastrum capricornutum* although still much less sensitive than aquatic invertebrates, with a NOAEL and LOAEL for growth rate inhibition of 0.077 and 0.15 mg/L, respectively. Decreased cell density was a somewhat more sensitive effect with a NOAEL 0.046 mg/L and a LOAEL of 0.077 mg/L (Hoberg 1992a).

In an aquatic microcosm study with mixed species of algae, Sundaram et al. (1997b) report that tebufenozide stimulated algal growth at concentrations of 0.25 and 0.75 mg/L.

4.1.3.5. Aquatic Microorganisms (Other than algae) – Other than the effect in algae, summarized in the previous section, no studies regarding the toxicity of tebufenozide to aquatic microorganisms were encountered.

4.2. EXPOSURE ASSESSMENT
4.2.1. Overview
Details of the exposure assessments for tebufenozide are given in the EXCEL workbook that accompany this risk assessment (Supplement 1). Most exposure assessments are based on two applications spaced 3 days apart at an application rate of 0.12 lb/acre. As in the human health risk assessment, two sets of exposure assessments are given for scenarios involving the longer-term consumption of contaminated vegetation: one for a single application at 0.12 lb/acre and another for two applications spaced 3 days apart at an application rate of 0.12 lb/acre.

Terrestrial animals might be exposed to any applied pesticide from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation. For tebufenozide, the highest acute exposure for a terrestrial vertebrate is associated with a fish-eating bird and could reach up to about 85 mg/kg. Exposures anticipated from the consumption of contaminated vegetation by terrestrial animals range from central estimates of about 0.15 mg/kg for a small mammal consuming fruit to about 3 mg/kg for a large bird with upper ranges of about 0.4 mg/kg for a small mammal and 9 mg/kg for a large bird. The consumption of contaminated water leads to much lower levels of exposure. A similar pattern is seen for chronic exposures. Estimated longer-term daily doses for the a small mammal from the consumption of contaminated vegetation at the application site are in the range of about 0.000002 mg/kg/day to 0.08 mg/kg/day. Large birds feeding on contaminated vegetation at the application site could be exposed to much higher concentrations, ranging from about 0.015 mg/kg/day to 11 mg/kg/day. The upper ranges of exposure from contaminated vegetation far exceed doses that are anticipated from the consumption of contaminated water, which range from about 0.0000003 mg/kg/day to 0.0002 mg/kg/day for a small mammal.

Exposure to aquatic organisms is based on essentially the same information used to assess the exposure to terrestrial species from contaminated water. The peak estimated concentration of tebufenozide in ambient water is 10 (0.005 to 40) µg/L after two applications of 0.12 lb/acre spaced three days apart. For longer-term exposures, the corresponding longer term concentrations in ambient water are estimated at about 0.004 (0.00002 to 0.01) µg/L.

4.2.2. Terrestrial Animals
Terrestrial animals might be exposed to any applied insecticide from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation.

In this exposure assessment, estimates of oral exposure are expressed in the same units as the available toxicity data. As in the human health risk assessment, these units are usually expressed as mg of agent per kg of body weight and abbreviated as mg/kg for terrestrial animals. One exception in this risk assessment involves terrestrial invertebrates. As detailed in the dose-response assessment (Section 4.3), toxicity data in units of mg/kg bw are available for some terrestrial invertebrates and these data are used in a manner similar to that for terrestrial vertebrates. For other species, however, standard toxicity studies report units that are not directly

useful in a quantitative risk assessments – e.g., contact toxicity based on petri dish exposures. As an alternative, some dose response assessments are based on field studies in which the dose metameter is simply the application rate in units of mass per area such as g a.i./ha.

For dermal exposures to terrestrial animals, the units of measure usually are expressed in mg of agent per cm^2 of surface area of the organism and abbreviated as mg/cm^2. In estimating dose, however, a distinction is made between the exposure dose and the absorbed dose. The *exposure dose* is the amount of material on the organism (i.e., the product of the residue level in mg/cm^2 and the amount of surface area exposed), which can be expressed either as mg/organism or mg/kg body weight. The *absorbed dose* is the proportion of the exposure dose that is actually taken in or absorbed by the animal.

The exposure assessments for terrestrial animals are summarized in Worksheet G01. As with the human health exposure assessment, the computational details for each exposure assessment presented in this section are provided as scenario specific worksheets (Worksheets F01 through F16b). Given the large number of species that could be exposed to insecticides and the varied diets in each of these species, a very large number of different exposure scenarios could be generated. For this generic risk assessment, an attempt is made to limit the number of exposure scenarios.

Because of the relationship of body weight to surface area as well as to the consumption of food and water, small animals will generally receive a higher dose, in terms of mg/kg body weight, than large animals will receive for a given type of exposure. Consequently, most general exposure scenarios for mammals and birds are based on a small mammal or bird. For mammals, the body weight is taken as 20 grams, typical of mice, and exposure assessments are conducted for direct spray (F01 and F02a), consumption of contaminated fruit (F03, F04a, F04b), and contaminated water (F05, F06, F07). Grasses will generally have higher concentrations of insecticides than fruits and other types of vegetation (Fletcher et al. 1994; Hoerger and Kenaga 1972). Because small mammals do not generally consume large amounts of grass, the scenario for the assessment of contaminated grass is based on a large mammal (Worksheets F10, F11a, and F11b). Other exposure scenarios for a mammals involve the consumption of contaminated insects by a small mammal (Worksheet F14a) and the consumption of small mammals contaminated by direct spray by a large mammalian carnivore (Worksheet F16a). Exposure scenarios for birds involve the consumption of contaminated insects by a small bird (Worksheet F14b), the consumption of contaminated fish by a predatory bird (Worksheets F08 and F09), the consumption by a predatory bird of small mammals contaminated by direct spray and the consumption by a large bird of contaminated grasses (F12, F13a, and F13b).

While a very large number of other exposure scenarios could be generated, the specific exposure scenarios developed in this section are designed as conservative screening scenarios that may serve as guides for more detailed site-specific assessments by identifying the groups of organisms and routes of exposure that are of greatest concern.

4.2.2.1. Direct Spray – In the broadcast application of any insecticide, wildlife species may be sprayed directly. This scenario is similar to the accidental exposure scenarios for the general public discussed in Section 3.2.3.2. In a scenario involving exposure to direct spray, the amount absorbed depends on the application rate, the surface area of the organism, and the rate of absorption.

For this risk assessment, three groups of direct spray exposure assessments are conducted. The first, which is defined in Worksheet F01, involves a 20 g mammal that is sprayed directly over one half of the body surface as the chemical is being applied. The range of application rates as well as the typical application rate is used to define the amount deposited on the organism. The absorbed dose over the first day (i.e., a 24-hour period) is estimated using the assumption of first-order dermal absorption. An empirical relationship between body weight and surface area (Boxenbaum and D'Souza 1990) is used to estimate the surface area of the animal. The estimates of absorbed doses in this scenario may bracket plausible levels of exposure for small mammals based on uncertainties in the dermal absorption rate.

Other, perhaps more substantial, uncertainties affect the estimates for absorbed dose. For example, the estimate based on first-order dermal absorption does not consider fugitive losses from the surface of the animal and may overestimate the absorbed dose. Conversely, some animals, particularly birds and mammals, groom frequently, and grooming may contribute to the total absorbed dose by direct ingestion of the compound residing on fur or feathers. Furthermore, other vertebrates, particularly amphibians, may have skin that is far more permeable than the skin of most mammals. Quantitative methods for considering the effects of grooming or increased dermal permeability are not available. As a conservative upper limit, the second exposure scenario, detailed in Worksheet F02a, is developed in which complete absorption over day 1 of exposure is assumed.

Because of the relationship of body size to surface area, very small organisms, like bees and other terrestrial invertebrates, might be exposed to much greater amounts of a pesticide per unit body weight compared with small mammals. Consequently, a third exposure assessment is developed using a body weight of 0.093 g for the honey bee (USDA/APHIS 1993) and the equation above for body surface area proposed by Boxenbaum and D'Souza (1990). Because there is no information regarding the dermal absorption rate of tebufenozide by bees or other invertebrates, this exposure scenario, detailed in Worksheet F02b, also assumes complete absorption over the first day of exposure. As noted above, exposures for other terrestrial invertebrates are based on field studies in which application rate is the most relevant expression of exposure. This is discussed further in Section 3.3 (Dose-Response Assessment) and Section 3.4 (Risk Characterization).

Direct spray scenarios are not given for large mammals. As noted above, allometric relationships dictate that large mammals will be exposed to lesser amounts of a compound in any direct spray scenario than smaller mammals.

4.2.2.2. Indirect Contact – As in the human health risk assessment (see Section 3.2.3.3), the only approach for estimating the potential significance of indirect dermal contact is to assume a relationship between the application rate and dislodgeable foliar residue.   Unlike the human health risk assessment in which transfer rates for humans are available, there are no transfer rates available for wildlife species. As discussed in Durkin et al. (1995), the transfer rates for humans are based on brief (e.g., 0.5 to 1-hour) exposures that measure the transfer from contaminated soil to uncontaminated skin. Wildlife, compared with humans, are likely to spend longer periods of time in contact with contaminated vegetation. It is reasonable to assume that for prolonged exposures an equilibrium may be reached between levels on the skin, rates of absorption, and levels on contaminated vegetation, although there are no data regarding the kinetics of such a process. The bioconcentration data on tebufenozide indicates that this compound will accumulate in the tissue of the fish. Thus, it is plausible that the absorbed dose resulting from contact with contaminated vegetation will be as great as those associated with comparable direct spray scenarios and possibly larger than those associated with the consumption of contaminated vegetation.

4.2.2.3. Ingestion of Contaminated Vegetation or Prey – Since tebufenozide will be applied to vegetation, the consumption of contaminated vegetation is an obvious concern and separate exposure scenarios are developed for acute and chronic exposure scenarios for a small mammal (Worksheets F04a and F04b) and large mammal (Worksheets F10, F11a, and F11b) as well as large birds (Worksheets F12, F13a, and F13b).

As discussed in Section 2.4, tebufenozide may be applied once or twice per season at an application rate of up to 0.12 lb/acre per application. In order to encompass the effects of both a single application per season and two applications per season, two sets of exposure assessments are given for the all scenarios involving the longer-term consumption of contaminated vegetation: one for a single application at 0.12 lb/acre and another for two applications spaced 3 days apart at an application rate of 0.12 lb/acre. For example, Worksheet 04bi presents the time-weighted average dose for a single application and Worksheet 04bii presents the time-weighted average dose for two applications spaced 3 days apart. This is also done for Worksheets F11a, F11b, F13a, and F13b. The calculation of the time-weighted average doses are identical to those used in the human health risk assessment (Section 3.2.3.6).

For the consumption of contaminated vegetation, a small mammal is used because allometric relationships indicate that small mammals will ingest greater amounts of food per unit body weight, compared with large mammals. The amount of food consumed per day by a small mammal (i.e., an animal weighing approximately 20 g) is equal to about 15% of the mammal's total body weight (U.S. EPA/ORD 1989). When applied generally, this value may overestimate or underestimate exposure in some circumstances. For example, a 20 g herbivore has a caloric requirement of about 13.5 kcal/day. If the diet of the herbivore consists largely of seeds (4.92 kcal/g), the animal would have to consume a daily amount of food equivalent to approximately 14% of its body weight [(13.5 kcal/day ÷ 4.92 kcal/g)÷20g = 0.137]. Conversely, if the diet of the herbivore consists largely of vegetation (2.46 kcal/g), the animal would have to consume a

daily amount of food equivalent to approximately 27% of its body weight [(13.5 kcal/day ÷ 2.46 kcal/g)÷20g = 0.274] (U.S. EPA/ORD 1993, pp.3-5 to 3-6). For this exposure assessment (Worksheet F03), the amount of food consumed per day by a small mammal weighing 20 g is estimated at about 3.6 g/day or about 18% of body weight per day from the general allometric relationship for food consumption in rodents (U.S. EPA/ORD 1993, p. 3-6).

A large herbivorous mammal is included because empirical relationships of concentrations of pesticides in vegetation, discussed below, indicate that grasses may have substantially higher pesticide residues than other types of vegetation such as forage crops or fruits (Worksheet A04). Grasses are an important part of the diet for some large herbivores, but most small mammals do not consume grasses as a substantial proportion of their diet. Thus, even though using residues from grass to model exposure for a small mammal is the most conservative approach, it is not generally applicable to the assessment of potential adverse effects. Hence, in the exposure scenarios for large mammals, the consumption of contaminated range grass is modeled for a 70 kg herbivore. Caloric requirements for herbivores and the caloric content of vegetation are used to estimate food consumption based on data from U.S. EPA/ORD (1993). Details of these exposure scenarios are given in worksheets F10 for acute exposures as well as Worksheets F11a and F11b for longer-term exposures.

For the acute exposures, the assumption is made that the vegetation is sprayed directly – i.e., the animal grazes on site – and that 100% of the animal's diet is contaminated. While appropriately conservative for acute exposures, neither of these assumptions are plausible for longer-term exposures. Thus, for the longer-term exposure scenarios for the large mammal, two sub-scenarios are given. The first is an on-site scenario that assumes that a 70 kg herbivore consumes short grass for a 90 day period after application of the chemical. In the worksheets, the contaminated vegetation is assumed to account for 30% of the diet with a range of 10% to 100% of the diet. These are essentially arbitrary assumptions reflecting grazing time at the application site by the animal. Because the animal is assumed to be feeding at the application site, drift is set to unity - i.e., direct spray. This scenario is detailed in Worksheet 11a. The second sub-scenario is similar except the assumption is made that the animal is grazing at distances of 25 to 100 feet from the application site (lowing risk) but that the animal consumes 100% of the diet from the contaminated area (increasing risk). For this scenario, detailed in Worksheet F12b, AgDRIFT is used to estimate deposition on the off-site vegetation. Drift estimates from AgDrift are summarized in Worksheet A06 and this model is discussed further in Section 4.2.3.2.

The consumption of contaminated vegetation is also modeled for a large bird. For these exposure scenarios, the consumption of range grass by a 4 kg herbivorous bird, like a Canada Goose, is modeled for both acute (Worksheet F12) and chronic exposures (Worksheets F13a and F13b). As with the large mammal, the two chronic exposure scenarios involve sub-scenarios for on-site as well as off-site exposure.

For this component of the exposure assessment, the estimated amounts of pesticide residue in vegetation are based on the relationship between application rate and residue rates on different

types of vegetation. As summarized in Worksheet A04, these residue rates are based on estimated residue rates from Fletcher et al. (1994).

Similarly, the consumption of contaminated insects is modeled for a small (10g) bird and a small (20g) mammal. No monitoring data have been encountered on the concentrations of tebufenozide in insects after applications of tebufenozide. The empirical relationships recommended by Fletcher et al. (1994) are used as surrogates as detailed in Worksheets F14a and F14b. To be conservative, the residue rates from small insects are used – i.e., 45 to 135 ppm per lb/ac – rather than the residue rates from large insects – i.e., 7 to 15 ppm per lb/ac.

A similar set of scenarios is provided for the consumption of small mammals by either a predatory mammal (Worksheet 16a) or a predatory bird (Worksheet 16a). Each of these scenarios assumes that the small mammal is directly sprayed at the specified application and the concentration of the compound in the small mammal is taken from the worksheet for direct spray of a small mammal under the assumption of 100% absorption (Worksheet F02a).

In addition to the consumption of contaminated vegetation and insects, tebufenozide may reach ambient water and fish. Thus, a separate exposure scenario is developed for the consumption of contaminated fish by a predatory bird in both acute (Worksheet F08) and chronic (Worksheet F09) exposures. Because predatory birds usually consume more food per unit body weight than do predatory mammals (U.S. EPA 1993, pp. 3-4 to 3-6), separate exposure scenarios for the consumption of contaminated fish by predatory mammals are not developed.

4.2.2.4. Ingestion of Contaminated Water – Estimated concentrations of tebufenozide in water are identical to those used in the human health risk assessment (Worksheet B06). The only major differences involve the weight of the animal and the amount of water consumed. There are well-established relationships between body weight and water consumption across a wide range of mammalian species (e.g., U.S. EPA 1989). Mice, weighing about 0.02 kg, consume approximately 0.005 L of water/day (i.e., 0.25 L/kg body weight/day). These values are used in the exposure assessment for the small (20 g) mammal. Unlike the human health risk assessment, estimates of the variability of water consumption are not available. Thus, for the acute scenario, the only factors affecting the estimate of the ingested dose include the field dilution rates (i.e., the concentration of the chemical in the solution that is spilled) and the amount of solution that is spilled. As in the acute exposure scenario for the human health risk assessment, the amount of the spilled solution is taken as 200 gallons. In the exposure scenario involving contaminated ponds or streams due to contamination by runoff or percolation, the factors that affect the variability are the water contamination rate, (see Section 3.2.3.4.2) and the application rate. Details regarding these calculations are summarized in Worksheets F06 and Worksheet F07.

4.2.3. Terrestrial Plants

Terrestrial plants will certainly be exposed to tebufenozide.   A large number of different exposure assessments could be made for terrestrial plants – i.e., direct spray, spray drift, runoff, wind erosion and the use of contaminated irrigation water. Such exposure assessments are

typically conducted for herbicides. For tebufenozide, however, the development of such exposure assessments would serve no purpose. As discussed in Section 4.1.2.4 (Hazard Identification for Terrestrial Plants), there is no basis for asserting that tebufenozide will cause adverse effects in terrestrial plants. Thus, no formal exposure assessment is conducted for terrestrial plants.

4.2.4. Soil Organisms

For both soil microorganisms and soil invertebrates, the toxicity data are typically expressed in units of soil concentration – i.e., mg agent/kg soil which is equivalent to parts per million (ppm) concentrations in soil.   The GLEAMS modeling, discussed in Section 3.2.3.4.3, provides estimates of concentration in soil as well as estimates of off-site movement (runoff, sediment, and percolation). Based on the GLEAMS modeling, concentrations in clay, loam, and sand over a wide range of rainfall rates are summarized in Table 4-1. As indicated in this table, peak soil concentrations after two applications at an application rate of 0.12 lb/acre are in a relatively narrow range: about 0.02 to 0.1 mg/kg (ppm) over all soil types and rainfall rates. Longer term concentrations in soil are all low and are on the order of 0.003 to 0.05 mg/kg – i.e., 3 ppb to 50 ppb.

4.2.5. Aquatic Organisms

The plausibility of effects on aquatic species is based on estimated concentrations of tebufenozide in water that are identical to those used in the human health risk assessment. As summarized in Table 3-5, the peak estimated concentration of tebufenozide in ambient water is 10 (0.005 to 40) µg/L after two applications of 0.12 lb/acre spaced three days apart. For longer-term exposures, the corresponding longer term concentrations in ambient water are estimated at about 0.004 (0.00002 to 0.01) µg/L.

4.3. DOSE-RESPONSE ASSESSMENT
4.3.1. Overview
The specific toxicity values used in this risk assessment are summarized in Table 4-2, and the derivation of each of these values is discussed in the various subsections of this dose-response assessment. The first column in Table 4-2 specifies the organism to which the toxicity value applies. The available toxicity data support separate dose-response assessments in six classes of organisms: terrestrial mammals, birds, nontarget terrestrial invertebrates, fish, aquatic invertebrates, and aquatic algae. Different units of exposure are used for different groups of organisms depending on how exposures are likely to occur and how the available toxicity data are expressed.

Tebufenozide is relatively non-toxic to mammals and birds. For mammals, the toxicity values used in the ecological risk assessment are identical to those used in the human health risk assessments: an acute NOAEL for reproductive toxicity of 1000 mg/kg and a chronic NOAEL of 1.8 mg/kg/day based on effects on the blood. For birds, the acute NOAEL for tebufenozide is taken as 2150 mg/kg from an acute oral study in which the dose was administered in capsules for 21-days. The longer term NOAEL is taken as 15 mg/kg/day from a standard reproduction study in bobwhite quail.

For terrestrial invertebrates, three types of data are used to characterize risks: a contact bioassay in the honey bee, a soil bioassay in earthworms, and field studies in which population level effects were monitored in insects. The standard contact bioassay in honey bees indicates an NOEC of 2500 mg/kg bw, comparable to the acute toxicity values in mammals and birds. The earthworm bioassay indicates a NOEC of 1000 mg/kg soil. The available field studies indicate that tolerant insect species are not affected by application rates up to 0.24 lb/acre. The true NOEC may be higher – i.e., an LOEC has not been identified for tolerant species of terrestrial insects. Conversely, application rates as low as 0.03 lb/acre have been shown to adversely affect sensitive nontarget insects, primarily *Lepidoptera* and a NOEC for sensitive species has not been identified.

Acute toxicity values for aquatic species indicate relatively little difference between fish and aquatic invertebrates. For fish, the acute NOEC values are 0.39 mg/L and 1.9 mg/L for sensitive and tolerant species, respectively.   For invertebrates, the corresponding acute NOEC values are 0.12 mg/L and 0.82 mg/L. Differences between fish and invertebrates are difficult to assess in terms of longer-term toxicity. For fish, data are available on only a single species, the fathead minnow, and only a LOAEL of 0.048 mg/L is available. For invertebrates, longer-term NOEC values of 0.0035 mg/L and 0.029 mg/L are used for sensitive and tolerant species. Toxicity values for aquatic plants are taken as 0.077 mg/L for sensitive species and 0.64 mg/L for tolerant species, somewhat below the acute NOEC values in fish and aquatic invertebrates. Because of the short life-cycle of individual algal cells, the relatively short-term bioassays in algae (i.e., 96 to 120 hours) are applied to both acute and longer-term concentrations for the characterization of risk.

4.3.2. Toxicity to Terrestrial Organisms

4.3.2.1. Mammals – As summarized in the dose-response assessment for the human health risk assessment (see Section 3.3.3.), the most sensitive effect in experimental mammals involves toxic effects in red blood cells. The chronic NOAEL for this endpoint in experimental mammals is 1.8 mg/kg/day (U.S. EPA 1999b) and is based on a dog study (Richards 1992a) in which beagles of either sex were provided with dietary concentrations of 0, 15, 50, 250, or 1500 ppm technical grade tebufenozide for 52 weeks (Appendix 2). No effects were seen in the 50 ppm exposure group which corresponded to an average dose of 1.8 mg/kg/day. At 250 ppm, which corresponded to an average dose of 20 mg/kg/day, a direct effect on red blood cells was indicated by increased concentrations of methemoglobin in the blood as well as changes in several other hematological parameters associated with toxic effects in red blood cells. Thus, for this risk assessment, 1.8 mg/kg/day is taken as the chronic NOAEL for general toxic effects.

Tebufenozide is also associated with adverse reproductive effects in mammals in a 2-generation study (see Section 3.1.4). In the study by Danberry et al. (1993), reproductive effects were not observed in rats given a dietary concentration of 150 ppm ($\approx$12 mg/kg bw) tebufenozide; however, in the same study, rats given a dietary concentration of 2000 ppm ($\approx$160 mg/kg bw) demonstrated clearly adverse effects, including increased mortality in females during delivery and decreases in implantation. This endpoint, with a longer-term NOAEL of 12 mg/kg/day and a LOAEL of 160 mg/kg/day, is also used in the characterization of risk (Section 4.4.2) to help elaborate the potential effects of exposures that exceed the general NOAEL of 1.8 mg/kg/day.

Consistent with the approach taken in the human health risk assessment (Section 3.3.4), acute (1-day) exposures will be based on the acute NOAEL of 1000 mg/kg/day from reproduction studies in both rats (Hoberman 1991) and rabbits (rabbits) involving 10 to 13 day exposure periods.

4.3.2.2. Birds – As detailed in Appendix 4, adverse reproductive effects were observed in bobwhite quail provided with dietary concentrations of 300 or 1000 ppm (Beavers et al. 1993b). Similar effects were not observed in mallard ducks provided with dietary concentrations of up to 1000 ppm in a study conducted by the same investigators (Beavers et al. 1993a) or in a follow-up study on bobwhite quail provided with dietary concentrations of up to 615 ppm (Reinert 1995a). As discussed in Section 4.1.2.2, the earlier study by Beavers et al. (1993b) is used to identify reproductive toxicity as an endpoint of concern in this risk assessment because there is no basis for discounting the study or explaining the discrepancies between the Beavers et al. (1993b) and Reinert (1995a) studies in bobwhite quail. In addition, reasonable consistency is apparent in the reported dose levels associated with reproductive effects in mammals and the reported dose levels in Beavers et al. (1993b) study. This approach is consistent with that taken by U.S. EPA (1999e).

It is worth noting that the two quail studies use different methods to report the estimated dose (i.e., the dose as mg/kg bw/day based on dietary concentrations and food consumption). In the study by Beavers et al. (1993b), "No attempt was made to quantify the amount of feed wasted by

the birds, as the wasted feed is normally scattered and mixed with water and excreta." (Beavers et al. 1993b, p. 16).   In the study by Reinert (1995a), food consumption estimates did explicitly consider measurements of food wastage (i.e., food scattered from the container and not consumed). Furthermore, the study by Beavers et al. (1993b) states explicitly that food was administered *ad libitum*—an excess of food was freely available to the animals. This protocol is not specified in the study by Reinert (1995a); however, it seems reasonable to assume that the food was available *ad libitum* because a restricted feeding protocol is atypical and would have been specified in the methods section of the study. These reporting differences are relatively inconsequential, assuming that both studies use *ad libitum* feeding.

Of greater importance, however, is the exposure metameter (i.e., how the exposure is expressed in the dose-response and the exposure assessments). The U.S. EPA (1999e) uses reported dietary concentrations. This approach, however, may be under protective. Laboratory diets generally involve the use of dry food, and dry food is specified in all of the bird feeding studies on tebufenozide. Dry laboratory chow usually has a higher caloric content than food consumed in the wild, if only because most food consumed in the wild has a high water content. In addition, most reported concentrations of a pesticide in environmental samples are given on a wet (natural) weight rather than a dry (dedicated) weight basis. Consequently, animals tend to eat greater amounts of food in the wild than they do under laboratory conditions (U.S. EPA 1993). Consequently, for a fixed concentration in food, ingested doses expressed as mg/kg bw/day often will be higher in free living animals than in laboratory animals.

Because of these relationships, Forest Service risk assessments use doses expressed as mg/kg body weight for both the exposure and dose-response assessments. As detailed in the worksheets, information on caloric requirements and caloric values of different foods are used to estimate the amount of a particular food that an animal will use.

For this risk assessment, the food consumption values reported by Beavers et al. (1993b) are used to estimate a NOAEL and a LOAEL of 15 and 45 mg/kg bw/day, respectively. This is not the most conservative approach that could be taken, because Beavers et al. (1993b) did not consider wastage in their estimates of food consumption. By comparison with the study by Reinert (1995a), the food consumption and hence the ingested amounts of tebufenozide could have been lower by a factor of about 2 [i.e., food consumption rates of 30 g per bird in Beavers et al. (1993b) and 16 g per bird in Reinert (1995a)]. Compared with other uncertainties in this risk assessment, this difference is relatively modest. The dose adjustment is incorporated explicitly into the dose-response assessment, and given further consideration in the risk characterization.

As with mammals, the acute toxicity of tebufenozide to birds appears to be very low. As indicated in Appendix 4, acute dietary LC_{50} values are greater than 5000 ppm (mg tebufenozide per kg diet) in both bobwhite quail and mallard ducks (Fletcher 1990a,b). In addition, 21 daily doses at both 1470 and 2150 mg a.i./kg bw, via gelatin capsule, caused no signs of toxicity in male or female bobwhite quail (Fletcher 1987). For this risk assessment, the 21-day exposure data from Fletcher (1987) will be used set an acute NOAEL of 2150 mg/kg bw for birds and this

value will be applied to all short-term (1-day) exposure assessments.

4.3.2.3. Terrestrial Invertebrates – As discussed in Section 4.1.2.3, tebufenozide mimics the invertebrate hormone 20-hydroxyecdysone and could cause adverse effects in a variety of terrestrial invertebrates. Notwithstanding this assertion, however, there are adequate field and field simulation studies clearly indicating that tebufenozide is much more toxic to *Lepidoptera* than to other insects.

Dose-response assessments for the effects of tebufenozide on terrestrial invertebrates could be based on either laboratory toxicity studies (Appendix 5) or field studies (Appendix 6). Most of the laboratory studies are on target rather than nontarget invertebrates and many involve exposures that are not readily applied to risk assessment. Studies that do involve both target and nontarget insects indicate that tebufenozide is more toxic to *Lepidoptera* (target species) than non-lepidopteran arthropods (Medina et al. 2002, 2003; Pietrantionio and Benedict 1999). In addition, tebufenozide appears to be less toxic to one nontarget species (lacewing) than diflubenzuron, another agent used to control the gypsy moth (Medina et al. 2002, 2003; Rumph et al. 1998).

The laboratory observations that non-lepidopteran arthropods are less sensitive to tebufenozide than *Lepidoptera* are supported by the field studies detailed in Appendix 6. A summary of the most relevant field studies is given in Table 4-3. In this table, efficacy studies summarized in Appendix 6 – i.e., those studies looking only at effects on target species, are omitted. Based on the study by Butler et al. (1997), both target and nontarget macrolepidoptera will be adversely affected at application rates as low as 0.03 lb/acre. Field studies at lower application rates have not been encountered and a NOAEL for nontarget macrolepidoptera cannot be identified. Similarly, a clear LOAEL for non-lepidopteran arthropods has not been identified. Mulder and Prescott (1999a) report a decrease in the numbers of beneficial arthropods on Day 3 after the application of tebufenozide at 0.125 lb a.i./acre but not at 0.24 lb a.i./acre. In addition, no effects on beneficial arthropods were seen at 0.125 lb/acre or 0.25 lb/acre on Day 5 to Day 15 after treatment.

For this risk assessment, the assumption is made that effects on sensitive nontarget *Lepidoptera* are likely to be comparable to those seen in target species. This assumption is based on the field study by Butler et al. (1997) in which a decrease in abundance in some lepidopteran species was noted after the application of Mimic 4F at rates of 0.03 and 0.06 lb a.i./acre. This may be a conservative assumption because, as noted by Butler et al. (1997), not all nontarget lepidopteran species were affected. Conversely, these investigators also noted that problems were encountered in the application of Mimic 4F, which resulted in poorer than expected efficacy. Thus, effects in nontarget lepidopteran species also may have been underestimated.

In the risk characterization, the minimum recommended application rate of 0.03 lb a.i./acre is taken as the exposure level that could be associated with adverse effects in some nontarget lepidopteran species. The true NOAEL in terms of application rate has not been defined for

nontarget lepidopteran species.

The potential for adverse effects on other nontarget insects is characterized quantitatively on the basis of the standard bioassay in the honey bee (Atkins. 1990; Chan 1995) in which no mortality was observed at doses of up to 233.98 µg a.i./bee or about 2500 mg/kg bw (see Section 4.1.2.3). As indicated in Table 4-2, this risk assessment also uses an application rate of 0.24 lb/acre as a functional NOEC for non-lepidopteran arthropods. This is based on the studies summarized in Table 4-3. As noted above, the application rate of 0.125 lb/acre from Mulder and Prescott (1999a) could be interpreted as a marginal LOEC. This interpretation would be grossly conservative because the effects seen at 0.125 lb/acre were transient and were not seen at 0.24 lb/acre.

Toxicity to soil invertebrates will be based on the standard toxicity bioassay in earthworms (Garvey 1992, discussed in Section 4.1.2.3) in which no effects were noted at soil concentrations of up to 1000 ppm (1000 mg/kg soil).

4.3.2.4. Terrestrial Plants and Microorganisms – As discussed in Sections 4.1.2.4. and 4.1.2.5., there is no reason to assume that tebufenozide will cause adverse effects in terrestrial plants or terrestrial microorganisms.   Nonetheless, no standard toxicity studies have been encountered that could be used to quantify risk in either terrestrial plants or soil microorganisms. Consequently, no dose-response assessment for these groups can be proposed.

4.3.3. Aquatic Organisms.
4.3.3.1. Fish – The acute bioassays on fish summarized in Appendix 7 provide estimates of exposures which might be associated acute effects in fish but only two species have been tested. The most sensitive species is the bluegill sunfish with a 96-hour LC_{50} of 3.0 (2.2 to 4.0) mg/L with an NOEC of 0.39 mg/L (Graves and Smith 1992b). Rainbow trout appear to be somewhat less sensitive, with an LC_{50} value of 5.7 mg/L (4.7 to 6.5 mg/L) and an NOEC of 1.9 mg/L (Graves and Smith 1992c). For this risk assessment, the NOEC values of 0.39 mg/L and 1.9 mg/L are used to assess the consequences of short-term exposures for sensitive and tolerant species.

The assessment of the effects of tebufenozide that might be associated with chronic exposure to contaminated ambient water from the normal use and application of this product is based on the full life cycle study in fathead minnows by Rhodes and Leak (1996) supported by the egg and fry study by Bettancourt (1992).

In the egg and fry study (Bettancourt 1992), eggs were incubated at mean measured concentrations of 0, 0.084, 0.14, 0.22, 0.36, or 0.71 mg a.i./L by continuous exposure for 35 days. Based on a comparison to pooled controls (i.e., untreated and solvent treated animals with a combined survival of 94%), Bettancourt (1992) reports no effects on survival at any concentration level. The U.S. EPA (1999e), however, classified the 0.71 mg/L concentration as an effect level based on decreased survival (88%) relative to survival in the solvent control

(98%). The U.S. EPA analysis was challenged by Rohm and Haas (Surprenant 1994).

In the full life cycle study (Rhodes and Leak 1996), newly hatched eggs were exposed to mean measured concentrations of 0, 0.048, 0.090, 0.18, 0.35, or 0.72 mg a.i./L, again using both untreated and solvent (acetone) controls. The exposure was continued for 219 days which allowed for full development of the fish and reproduction. The most sensitive endpoint reported by Rhodes and Leak (1996) using pooled control data was survival with a LOAEL of 0.35 mg a.i./L and a NOAEL of 0.18 mg a.i./L. Again using solvent control rather than pooled control data, the U.S. EPA identified the most sensitive effect as decreased eggs/spawn and identified the LOAEL as 0.048 mg a.i./L, the lowest concentration tested. Because the U.S. EPA does not consider that this study identified a NOAEL, the U.S. EPA stated that the full life cycle study must be repeated (U.S. EPA 1999e). Again, the U.S. EPA analysis was contested by Rohm and Haas (Reinert et al. 1999).

The decision to pool or not pool control data is both statistical and judgmental, and the discussion provided by Reinert et al. (1999) is reasonably complete and objective. It is worth noting, nonetheless, that the statistical re-analysis presented by Reinert et al. (1999) does indicate that the dose-response relationship for eggs/spawn has p values of 0.077 or 0.058, depending on whether standard or weighted regression is used. Although these values may be classified as 'insignificant' using the standard cutoff p value of 0.05, the selection of this or any other p value is itself judgmental.

The statistical analyses of these studies are open to reasonable debate; however, the Forest Service attempts to maintain a consistency with the U.S. EPA unless there is a compelling reason to do otherwise. For this risk assessment, there appears to be no compelling reason to deviate from the U.S. EPA assessment. Notwithstanding the reasonable arguments put forth by Reinert et al. (1999), the effect of tebufenozide on eggs/spawn is at least marginally significant. Furthermore, the use of solvent control data leads to more conservative assessments of risk in both the egg and fry study as well as the full life cycle study. While this may be coincidental, the consistency between the two studies suggests that the differences could be related to some factor that is not fully understood at this time. Consequently, this risk assessment treats 0.048 mg/L, the lowest concentration tested in the full life cycle study, as a LOAEL for fish reproduction.

For this risk assessment, a LOAEL of 0.048 mg/L is adopted for chronic effects in fish. This interpretation of the study is identical to that of the U.S. EPA (1999e). The data are not sufficient to propose separate values for tolerant and sensitive species.

4.3.3.2. Aquatic Invertebrates – Although data on the effects of tebufenozide on aquatic invertebrates is limited to three species (i.e, daphnids, midge larvae and lobsters as summarized in Appendix 8), variability is apparent regarding the acute toxicity of tebufenozide to aquatic invertebrates. Based on the available bioassays, the most sensitive species is the midge (*Chironomus riparius*) with an acute LC_{50} of 0.3 mg/L and an NOEC of 0.12 mg/L (van der Kolk 1997). Daphnids appear to be much more tolerant, with an LC_{50} value of 3.8 mg/L and a

corresponding NOEC of 0.82 mg/L (Graves and Smith 1992a). The apparent high sensitivity of midge relative to *Daphnia* may be related to differences in the types of bioassays that are run on midges (sediment assays) compared to those run on *Daphnia* (water only without sediment). The highest reported NOEC in lobsters is 0.1 mg/L (Dionne 1998). Because the study on lobsters was conducted at very low concentrations and no effects were seen at any concentration, there is no basis for asserting that lobsters are sensitive species. For this risk assessment, the acute NOEC values of 0.12 mg/L and 0.82 mg/L are used to assess the consequences of short-term exposures for sensitive and tolerant species of aquatic invertebrates.

The midge is the most sensitive species for assessing the potential effects of chronic exposure. In the study by van der Kolk (1997), a concentration of 0.0053 mg/L caused a decrease in the larval emergence rate, and a concentration of 0.04 mg/L caused complete suppression of larval emergence. The NOAEL in this study is 0.0035 mg/L. Based on a standard 21-day reproductive study, *Daphnia magna* are substantially less sensitive with a reproductive NOEC of 0.029 mg/L and a corresponding LOEC of 0.059 mg/L (McNamara 1991). For this risk assessment, the longer-term NOEC values of 0.0035 mg/L and 0.029 mg/L are used to assess the consequences of longer-term exposures for sensitive and tolerant species of aquatic invertebrates.

4.3.3.3. Aquatic Plants – As with fish and invertebrates, the available studies (Section 4.3.3.4 and Appendix 8) suggest substantial differences in sensitivity among species of freshwater algae. For this risk assessment, risks to sensitive species are characterized using the lowest reported NOEC for algal growth of 0.077 mg/L in *Scenedesmus subspicatus* from the study by (Hoberg 1992a). An over eight-fold higher NOEC of 0.64 mg/L has been reported for *Selenastrum capricornutum* (Reinert 1993b) and this value will be used to characterize risks in tolerant algal species. Although these tests are conducted for relatively short periods of time (i.e., 96 to 120 hours), these NOEC values are applied to both acute and longer-term concentrations because of the short life-cycle of individual algal cells.

4.3.3.4. Aquatic Microorganisms – Other than the information on algae provided above, there are no data regarding the toxicity of tebufenozide to aquatic microorganisms. Accordingly, no dose-response assessment is possible for this group.

4.4. RISK CHARACTERIZATION
4.4.1. Overview
The use of tebufenozide to control the gypsy moth may result in adverse effects in nontarget *Lepidoptera* but there is little indication that other species will be impacted under normal conditions of use even at the highest application rate. Tebufenozide is an insecticide that is effective in controlling populations of lepidopteran pests. No data, however, are available on toxicity to nontarget *Lepidoptera*. For this risk assessment, the assumption is made that nontarget *Lepidoptera* may be as sensitive to tebufenozide as target *Lepidoptera*. Thus, adverse effects in nontarget *Lepidoptera* would be expected after applications that are effective for the control of lepidopteran pest species.

There is no indication that short term exposures to tebufenozide will cause direct adverse effects in any terrestrial vertebrates or non-lepidopteran invertebrates even at the upper range of plausible exposures as well as accidental exposures. Similarly, direct adverse effects from longer term exposures in birds and mammals appear to be unlikely under most conditions. In some extreme cases, exposures in some large mammals could exceed the NOEC but the exposures would be below levels that have been associated with frank signs of toxicity. Effects on birds due to a decrease in available prey – i.e., terrestrial invertebrates – may be plausible. Adverse effects in aquatic species are not expected under normal conditions of use. In the case of a large accidental spill into a relatively small body of water, however, adverse effects could be expected in aquatic vertebrates, invertebrates, and plants.

4.4.2. Terrestrial Organisms
4.4.2.1. Terrestrial Vertebrates – The risk characterization for terrestrial vertebrates is summarized in Worksheet G02 for the maximum application rate of 0.12 lb/acre. The risk characterization is based on the estimates of exposure summarized in Section 4.2.3 and the toxicity values for diflubenzuron derived in Section 4.3.2.1 and summarized in Table 4.2. For most exposure scenarios, hazard quotients are included for both single applications and two applications spaced three days apart. For those exposure scenarios that do not include both single and double applications, the exposures are based on two applications

None of the acute exposures result in hazard quotients that exceed the level of concern. The highest acute hazard quotient for any vertebrate is 0.04 – i.e., the consumption of contaminated fish by a fish-eating bird after an accidental spill – and this is below the level of concern by a factor of 20. Other more plausible exposure scenarios such as the consumption of contaminated vegetation and water are in the range of 0.000006 to 0.008, below the level of concern by factors of 125 to about 160,000.

Similarly, for longer term exposures, central and lower estimates of hazard quotients are substantially below a level of concern. The highest central estimate for any hazard quotient is 0.1 – i.e., below the level of concern by a factor of 10. At the upper ranges of exposure, however, the hazard quotient exceeds a level of concern for the consumption of contaminated vegetation on-site by a large mammal after either a single application (HQ=2) or two applications (HQ=4).

As noted in the dose response assessment for mammals, the hazard quotients for mammals are based on a NOAEL of 1.8 mg/kg/day from the study by Richards (1992a) in which the corresponding LOAEL – based on toxic effects in the blood – of 20 mg/kg/day. Thus, a hazard quotient of 11 [20 mg/kg/day ÷ 1.8 mg/kg/day] would suggest a high likelihood of adverse effects in blood. The estimated hazard quotients of 2 to 4 are below this level where adverse effects would be expected but some changes in blood could occur although the toxicologic significance of these effects would most likely be marginal because the 20 mg/kg/day dose group in the study by Richards (1992a) did not display any overt signs of toxicity. Another factor to consider in interpreting these risk quotients is the proportion of the animal's diet that is contaminated. The risk quotients for the consumption of contaminated vegetation that exceed the level of concern are all based on the assumption that 100% of the animal's diet is contaminated. In other words, the animal consumes only vegetation that has been directly sprayed with tebufenozide. Thus, the potential impact of canopy interception is not considered.

As discussed in Section 4.1.2.2 and detailed further in Appendix 6, the field study by Holmes (1998) noted suggestive effects on reproductive performance in Tennessee warblers – i.e., a decrease in the average number of eggs per nest and percent of eggs hatching. In addition, female warblers evidenced a decrease in brooding time and increase in foraging times, suggesting a decrease in prey availability. While the effects were not statistically significant, this study suggests that some birds may be impacted through a decrease in available prey secondary to the effects of tebufenozide on terrestrial invertebrates, as discussed further in Section 4.4.2.2.

The verbal interpretation of these risk quotients is thus somewhat uncertain. There is no indication that short term exposures to tebufenozide will cause adverse effects in any terrestrial vertebrates even at the upper range of plausible exposures as well as accidental exposures. Similarly, adverse effects from longer terms exposures in birds and mammals appears to be unlikely under most conditions. In some extreme cases, exposures in some large mammals could exceed the NOEC but the exposures would be below the known LOEC.

4.4.2.2. Terrestrial Invertebrates – Tebufenozide is an insecticide that is effective in controlling populations of lepidopteran pests. No data, however, are available on toxicity to nontarget *Lepidoptera*. For this risk assessment, the assumption is made that nontarget *Lepidoptera* may be as sensitive to tebufenozide as target *Lepidoptera*. Thus, applications of 0.03 lb/acre are considered a LOEC based on the studies summarized in Table 4-3. As noted in Section 4.3.2.3, a NOEC for target and nontarget *Lepidoptera* cannot be identified. The USDA may use application rates as low as 0.015 lb/acre and these applications are presumably effective in the control of the gypsy moth. Under the assumption that nontarget *Lepidoptera* are as sensitive to tebufenozide as target species, adverse effects in nontarget *Lepidoptera* would be expected.

Adverse effects in other insect species do not appear to be likely based on either the standard toxicity study in bees or the available field studies. As indicated in Worksheet G01, the hazard quotient for the direct spray of a bee is 0.08 at the maximum application rate of 0.12 lb/acre.

Based on field studies, application rates of up to 0.24 lb/acre appear to have no adverse effect on beneficial arthropods. Using application rates, the highest hazard quotient would be 0.5 [0.12 lb/acre ÷ 0.24 lb/acre]. Because effects on beneficial arthropods have not be examined at higher applications rates, the true NOEC for beneficial arthropods may be higher and perhaps substantially higher than 0.24 lb/acre. Consequently, the hazard quotient of 0.5 based on application rates is not inconsistent with the hazard quotient of 0.08 based on the honey bee toxicity bioassay.

Toxicity data are also available on earthworms in which no effects were noted at soil concentrations of up to 1000 ppm (1000 mg/kg soil) (Section 4.3.2.3). As noted in Table 4-1, the peak concentration that would be expected in soil after two applications at a rate of 0.12 lb/acre is about 0.1 ppm, below the level of concern by a factor of 10,000.

Thus, while the available data on nontarget terrestrial invertebrates are limited, it seems reasonable to assert that effected on nontarget lepidopterans are plausible at application rates that are effective in the control of target lepidopterans such as the gypsy moth. There is no basis for asserting that effects on other nontarget arthropods or other terrestrial invertebrates are plausible.

4.4.2.3. Terrestrial Plants and Microorganisms – No quantitative risk assessment to terrestrial plants is made for tebufenozide. As discussed in Section 4.1.2.4, there are no data on the toxicity of this compound to either terrestrial plants or microorganisms. This lack of data, however, adds no substantial uncertainty to this risk assessment. Tebufenozide has been extensively tested in both the laboratory and field studies for efficacy in the protection of terrestrial plants from insect pests. If tebufenozide were toxic to plants at applications at or substantially above those used to control the gypsy moth, it is likely that reports of such phytotoxicity would be noted. No such reports have been encountered.

4.4.3. Aquatic Organisms

A summary of the risk quotients for aquatic organisms is presented in worksheet G03. Risk characterizations are presented for sensitive and tolerant species of aquatic organisms (vertebrates, invertebrates, and plants) for three exposure scenarios (an accidental spill, expected peak concentrations, and expected longer term concentrations of tebufenozide in water). The expected peak and longer term concentrations are summarized in Table 3-5 and discussed in Section 3.2.3.4.6. The concentrations associated with an accidental spill are calculated in Worksheet D05 and discussed in Section 3.2.3.4.1. The toxicity values used for each group of organisms are summarized in Table 4-2 and discussed in Section 4.3.

The risk characterizations for each group of aquatic organisms are essentially identical. Under normal conditions of use at the highest anticipated application rate, no effects are expected in any group of organisms: vertebrates, invertebrates, or plants. In the case of an accidental spill, however, adverse effects would be expected in each group of organisms.

4.4.3.1. Aquatic Vertebrates – Under normal conditions of use, the highest hazard quotient for

sensitive species of fish is 0.1 – the hazard quotient associated with expected peak concentrations in water at the maximum anticipated application rate. The upper range of longer term concentrations in water are below a level of concern by a factor of about 33 (HQ=0.03). In the case of an accidental spill, however, the central estimate and the upper range of the hazard quotients exceeds a level of concern for both sensitive and tolerant species. As discussed in 3.2.3.4.1, the accidental spill scenario is both extreme and arbitrary, involving the spill of a relatively large amount of chemical into a small body of water.

4.4.3.2. Aquatic Invertebrates – Based on expected concentrations of tebufenozide in water under normal conditions of use, the upper ranges of the hazard quotients for sensitive aquatic invertebrates are 0.3 for short term peak concentrations and 0.4 for longer term concentrations. While these hazard quotients are somewhat higher than the corresponding hazard quotients for aquatic vertebrates, they are below a level of concern.   In the case of an accidental spill, the concentrations in water exceed the level of concern for both sensitive and tolerant species of aquatic invertebrates.

4.4.3.3. Aquatic Plants – The risk characterization for aquatic plants is based on bioassay data using algae. Because bioassay on algae are conducted only over relatively short periods of time – i.e., 96 to 120 hours – the toxicity values for both tolerant and sensitive species of algae are all essentially short term. As with both aquatic vertebrates and invertebrates, none of the expected concentrations in water exceed the level of concern for sensitive or tolerant species of algae even at the upper ranges of plausible exposures. Also as with aquatic vertebrates and invertebrates, the level of concern is exceed for both sensitive and tolerant species of algae in the case of an accidental spill.

5. REFERENCES

Addison JA. 1996. Safety testing of tebufenozide, a new molt-inducing insecticide, for effects on nontarget soil invertebrates. Ecotoxicol Environ Safety. 33(1): 55-61.

Anderson D; Shuey D. 1994. Delayed Contact Hypersensitivity Study in Guinea Pigs: Mimic 240 LV Insecticide: Final Report: Lab Project Number: 94P-160: 94R-160. Unpublished study prepared by Rohm and Haas Company. MRID No.44727708. 38 pp.

Aso S. 1995. Two Generation Reproduction Study of RH-5992 in Rats: Final Report: Lab Project Number: 93P/101: 93RC/101. Unpublished study prepared by Hita Research Lab. MRID No.43797701. 360 pp.

Atkins E. 1990. RH-5992 Technical: Bee Adult Toxicity Dusting Test: Lab Project Number: BATDT 445/446: SUMM. 795: 87RC-0015. Unpublished study prepared by Univ. of Cal., Dept. of Entomology, Inc. MRID No.42436244. 57 pp.

Beavers J; Ross T. Jaber M. 1993a. RH-5992 Technical: A One Generation Reproduction Study with the Mallard (*Anas platyrhynchos*): Lab Project Number: 129-148: 90RC-0222: 91P-222. Unpublished study prepared by Wildlife International Ltd. MRID No.42991503. 161 pp.

Beavers J; Ross T; Jaber M. 1993b. RH-5992 Technical: A One-Generation Reproduction Study with the Bobwhite (*Colinus virginianus*): Lab Project Number: 129-147: 90RC-0267: HWA 417-481. Unpublished study prepared by Wildlife International. MRID No.42991501. 165 pp.

Beeson DR; Lewis MC; Powell JM; Nimmo DR. 1998. Effect of pollutants on freshwater organisms. Water Environment Research. 70(4): 921-931.

Bemacki H; Ferguson J. 1994a. Acute Inhalation Toxicity Study in Rats: Final Report: RH-5992 70 WP: Lab Project Number: 94P-099: 94R-099. Unpublished study prepared by Rohm and Haas Co. MRID Nos.44200306. 26 pp.

Bemacki H; Ferguson J. 1994b. Mimic 240 LV Acute Inhalation Toxicity Study in Rats: Report No. 94R-161. Unpublished study prepared by Rohm and Haas Co. MRID No.44727705c. 43 pp plus proprietary Appendix.

Bettancourt M. 1992. RH-5992 Technical: Toxicity to Fathead Minnow (*Pimephales promelas*) Embryos and Larvae: Final Report: Lab Project Number: 91-9-3923: 86.0291.6133.120: 90RC-0212. Unpublished study prepared by Springbo. MRID No.42436242. 80 pp.

Biddinger DJ; Hull LA 1995. Effects of several types of insecticides on the mite predator, *Stethorus punctum* (Coleptera: Coccinellidae), including insect growth regulators and abamectin. J. Econ. Entomol. 88(2): 358-366.

Biddinger DJ; Hull LA; Rajotte EG 1998. Stage specificity of various insecticides to tufted apple bud moth larvae (Lepidoptera: Cidae). J. Econ. Entomol. 91(1): 200-208.

Biddinger DJ; Hull LA. 1999. Sublethal effects of selected insecticides on growth and reproduction of a laboratory susceptible strain of tufted apple bud moth (Lepidoptera: Tortricidae). Journal of Economic Entomology. 92 (2): 314-324.

Black C. 1992. RH 75,992: Microbial Mutagenicity Assay: Lab Project Number: 86P-035: 86R-013A. Unpublished study prepared by Rohm & Haas Co. MRID No.42436229. 13 pp.

Borland C; Harmes K; Cracknell N; Mack D; Higenbottam T. 1985. Methemoglobin levels in smokers and non-smokers. Arch. Environ. Health. 40(6): 330-333.

Boxenbaum J; D'Souza R. 1990. Interspecies pharmacokinetic scaling, biological design and neoteny. Adv. Drug Res. 19: 139-195.

Bradberry SM. 2003. Occupational methaemoglobinaemia. Mechanisms of production, features, diagnosis and management including the use of methylene blue. Toxicol Rev. 22(1):13-27.

Brown JJ. 1996. The compatibility of tebufenozide with a laboratory lepidopteran host-hymenopteran parasitoid population. Biol. Control. 6(1): 96-104.

Burnmaster DE. 1998. Lognormal distribution for total water intake and tap water intake by pregnant and lactating women in the United States. Risk Analysis. 18(5): 215-21.

Butler L; Kondo V; Blue D 1997. Effects of tebufenozide (RH-5992) for gypsy moth (Lepidoptera: Lymantriidae) suppression on nontarget canopy arthropods. Environ. Entomol. 26 (5):1009-1015.

C&P Press (Chemical and Pharmaceutical Press). 2004. Product Labels and Material Safety Data Sheets for Mimic 2LV, Confirm 2F, and Confirm TO. Available at: http://www.greenbook.net/. Downloaded January 30, 2004.

Cadogan BL; Retnakaran A; Meating JH 1997. Efficacy of RH5992, A new insect growth regulator against spruce budworm (Lepidoptera: Cidae) in a boreal forest. J. Econ. Entomol. 90(2): 551-559.

Cadogan BL; Thompson D; Retnakaran A; Scharbach RD; Robinson A; Staznik B. 1998. Deposition of aerially applied tebufenozide (RH5992) on balsam fir (*Abies balsamea*) and its control of spruce budworm (*Choristoneura fumiferana* (Clem.). Pesticide Science. 53(1): 80-90.

Calabrese EJ. 1991. Principles of animal extrapolation. Lewis Publishers. Chelsea, MI. Cited in USDA 1995.

Calabrese EJ; Baldwin LA. 1993. Performing Ecological Risk Assessments. Lewis Publishers, Boca Raton, LA, pp. 12-24.

Canadian Council of Forest Ministers. 1999. National Forestry Database Program. www.nrcan.gc.ca/cfs/proj/iepb/nfdp/cp95/data_e/ com95e.ht.

Carson WG; Kund GS; Trumble JT. 1999. Effect of insecticides on tomato insects, Saxena, K. N. (Ed.). Arthropod Management Tests, Vol. 24. V+478p. Entomological Society of America: Lanham, Maryland, USA. ISBN 0-938522-86-8; 24 (0): 176-178.

Centa A; Camera G; Zucchinetti P; Di Pietro P. 1985. Methemoglobinemia in the newborn and nursing infant: Genetic and acquired forms. Pathologica. 77(1052): 659-665.

Chan P. 1995. The Toxicity of Tebufenozide to Honey Bees: (Supplemental Data to MRID 42436244): Lab Project Number: 95R/1053. Unpublished study prepared by Rohm and Haas Co. MRID No.43797702. 127 pp.

Clay,H. 1992. RH 5992: 90-Day Oral (Dietary Administration) Toxicity Study in the Beagle: Lab Project Number: 616/11: 6660-616/11: 90RC-062. Unpublished study prepared by Hazleton UK. MRID No.42436223. 355 pp.

Consoli FL; Parra J RP; Hassan SA. 1998. Side-effects of insecticides used in tomato fields on the egg parasitoid *Trichogramma pretiosum* Riley (Hym., trichogrammatidae), a natural enemy of *Tuta absoluta* (Meyrick) (Lep., gelechiidae). Journal of Applied Entomology. 122(1): 43-47.

Cook J. 2004 Supervisory Entomologist, Gypsy Moth EIS Project Leader. 180 Canfield Street, Morgantown, WV 26505. Email to Patrick Durkin, SERA Inc. dated March 1, 2004 concerning application rates for Mimic in Forest Service programs.

Cress D. 1996. RH-75,992: Lack of Activity in a Yeast Estrogen Receptor Assay: Lab Project Number: 96R-1033. Unpublished study prepared by Rohm and Haas Co. MRID No.44080902. 6 pp.

Crisp TM; Clegg ED; Cooper RL; Wood WP; Anderson DG; Baetcke KP; Hoffmann JL; Morrow MS; Rodier DJ; Schaeffer JE; Touart LW; Zeeman MG; Patel YM. 1998. Environmental endocrine disruption: an effects assessment and analysis. Environmental Health Perspectives. 106(Suppl. 1): 11-56.

C&P Press (Chemical and Pharmaceutical Press). 2004. Greenbook.net. Product Labels and Material Safety Data Sheets for Dimilin 4L, Dimilin 25W, and other formulations of diflubenzuron. Available at: http://www.greenbook.net.

Calabrese, EJ. 1991. Principles of animal extrapolation. Lewis Publishers. Chelsea, MI.

Calabrese EJ; Baldwin LA. 1993. Performing Ecological Risk Assessments. Lewis Publishers, Boca Raton, LA, pp. 12-24.

Daly JS; Hultquist DE; Rucknagel DL. 1983. Phenazopyridine induced methemoglobinemia associated with decreased activity of erythrocyte cytochrome b5 reductase. J. Med. Gen. 20(4): 307-309.

Danberry T.; Romanello A.; Donofrio K.; et al. 1993. RH-5992: Two-Generation Reproduction Study in Rats: Amended Final Report: Lab Project Number: 90P202: 90R202A. Unpublished study prepared by Rohm and Haas Co. MRID No.42931207. 581 pp.

Das Gupta A; Vaidya MS; Bapat JP; Pavri RS; Baxi AJ; Advani SH. 1980. Associated red cell enzyme deficiencies and their significance in a case of congenital enzyomopenic methemoglobinemia. Acta. Haematol. (Basel). 64(5): 285-288.

De Sapio R. 1976. Calculus for the Life Sciences. W.H. Freeman and Company, San Francisco, California. 740 pp.

Dhadialla TS; Carlson GR; Le DP 1998. New Insecticides with ecdysteroidal and juvenile hormone activity. Annu. Rev. Entomol. 43: 545-569.

Dionne E. 1998. Confirm 2F--Acute Toxicity to Northern Lobsters (*Homarus americanus*) Under Static Conditions: Final Report: Lab Project Number: 98-11-7561: 86.1098.6226.585: 98RC-0210. Unpublished study prepared by. MRID No.44945701.

Dong L; Hawkins D. 1993. Dynamic Bioconcentration Study of RH-5992 with Bluegill Sunfish Metabolite Identification Phase: Lab Project Number: 34-93-45: TR34-93-45: 92-7-4342. Unpublished study prepared by Rohm and Haas Co. MRID No.42931224. 497 pp.

Dong L; Hazelton A; Fernandes J et al. 2000. Confirm 2F and Confirm 70WSP Agricultural Insecticides Reduced Risk Rationale-Citrus: Lab Project Number: 00R-1029. Unpublished study prepared by Rohm and Haas Company. 134 p. MRID 45141001.

Dorschner K.; Breuninger K. 1996. Tebufenozide: Magnitude of the Residue on Blueberry: Lab Project Number: IR-4 PR NO.06407: 06407.96-NC12: 06407.96-FL33. Unpublished study prepared by Del Monte Research Center, Horticultural Crops R. MRID No.44570501.

Dorschner K. 2001. Tebufenozide: Magnitude of the Residue on Sweet Potato: Lab Project Number: 06512: 06512.97-DEL03: 06512.97-NJ30. Unpublished study prepared by Del Monte Research Center. 348 p. MRID 45572101.

Dorschner K. 2002. Tebufenozide: Magnitude of the Residue on Grape: Lab Project Number: 06763: 06763.98-DEL07: 06763.98-NY21. Unpublished study prepared by Del Monte Research Center. 309 p. MRID 45612601.

Dunning JB. 1993. CRC Handbook of Avian Body Masses. CRC Press, Boca Raton FL, 371 pp.

Durkin PR. 1994. Comparison and Summary of Human Health Risk Assessments for the USDA Control and Eradication Programs. pp. 170-182 in: Proceedings of the 1994 Annual Gypsy Moth Review, D.H. Hilburn, K.J.R. Johnson, and A.D. Mudge (eds), U.S. Department of Agriculture, Salem, Oregon.

Durkin PR; Diamond G. 2002. Neurotoxicity, Immunotoxicity, and Endocrine Disruption with Specific Commentary on Glyphosate, Triclopyr, and Hexazinone: Final Report. SERA TR 01-43-08-04a dated January 30, 2002. Available at www.fs.fed.us/foresthealth/pesticide/risk.htm.

Durkin PR; Rubin L; Withey J; Meylan W. 1995. Methods of assessing dermal absorption with emphasis on uptake from contaminated vegetation. Toxicol. Indust. Health. 11(1): 63-79.

Ecobichon DJ. 1998. Occupational Hazards of Pesticide Exposure – Sampling, Monitoring, Measuring. Taylor & Francis, Philadelphia, PA. 251 pp.

Fleming MW. 1998. In vitro growth of swine roundworm larvae, Ascaris suum: cultivation techniques and endocrine regulation. J Helminthol Soc Wash 65(10):69-73. Summarized in Keller and Brown 1998a.

Fletcher D. 1987. 21-Day Acute Oral Toxicity Study with RH-5992 Technical in Bobwhite Quail: Lab Project Number: 87RC-0024: 87P-032: BLAL 87 QD 86. Unpublished study prepared by Bio-Life Assoc. MRID No.42436234. 27 pp.

Fletcher D. 1990a. 8-Day Dietary LC50 Study with RH-5992 Technical in Bobwhite Quail: Final Report: Lab Project Number: 87RC-0054: 87P-211: BLAL-87 QC 92. Unpublished study prepared by Bio-Life Assoc. MRID No.42436235. 34 pp.

Fletcher D. 1990b. 8-Day Dietary LC50 with RH-5992 Technical in Mallard Ducklings: Lab Project Number: 87RC-0055]: 87P-210: BLAL 87 DC 93. Unpublished study prepared by Bio-Life Asso. MRID No.42436237. 33 pp.

Fletcher D. 1990c. 8-Day Dietary LC50 Study with RH-5992 in Mallard Ducklings: Lab Project Number: 89P-99; 89RC-099; HLA 6228-107. Unpublished study prepared by Sitek Research Labs. 33 p. MRID 42436233.

Fletcher JS; Nellessen JE; Pfleeger TG. 1994. Literature review and evaluation of the EPA food-chain (Kenega) nomogram, an instrument for estimating pesticide residues on plants. Environ. Toxicol. Chem. 13(9):1383-1391.

Fussell W. 2004. USDA/APHIS. Email to Joseph Cook, USDA/FS, dated March 1, 2004 concerning application rates for Mimic in APHIS programs.

Garvey N. 1992. RH-5992 Technical--Subacute (14-Day) Toxicity to Earthworms (*Eisenia foetida*): Final Report: Lab Project Number: 91-11: 4003: 90RC-0211. Unpublished study prepared by Springborn Labs, Inc. MRID No.42991511. 62 pp.

Gingrich S.; Parno J. 1994. Eye Irritation Study in Rabbits: Mimic 240 LV Insecticide: Final Report: Lab Project Number: 94P-164: 94R-164. Unpublished study prepared by Rohm and Haas Company. MRID No.44727706. 19 pp.

Glaza S. 1993. Dermal Sensitization Study of RH-5992 Technical in Guinea Pigs--Maximization Test: Final Report: Lab Project Number: HWI 20800744: 92RC-101: 92P-101. Unpublished study prepared by Hazleton Wisconsin,. MRID No.42991506. 48 pp.

Goldstein A; Aronow L; Kaman SM. 1974. Principles of Drug Action: The Basis of Pharmacology. 2nd ed. John Wiley and Sons, New York, NY. 854 p.

Graves W.; Smith G. 1992a. RH-5992 Technical: A 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*): Final Report: Lab Project Number: 129A-109: 90RC-0065. Unpublished study prepared by Wildlife Intl. Ltd. MRID No.42436241. 122 pp.

Graves W; Smith G. 1992b. RH-5992 Technical: A 96-Hour Static Acute Toxicity Test with the Bluegill (*Lepomis macrochirus*): Final Report: Lab Project Number: 129A-108: 90RC-0063. Unpublished study prepared by Wildlife Intl. Lt. MRID No.42436239. 123 pp.

Graves W; Smith, G. 1992c. RH-5992 Technical: A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorrhynchus mykiss*): Lab Project Number: 129A-110: 90RC-0064. Unpublished study prepared by Wildlife Intl. Ltd. MRID No.42436240. 123 pp.

Gudi R. 1992. RH-5992 Technical: Acute Test for Chemical Induction of Chromosome Aberration in Rat Bone Marrow Cells In Vivo: [Final Report]: Lab Project Number: 0173-1531: 90RC-203. Unpublished study prepared by S. MRID No.42436231. 58 pp.

Gurr GM; Thwaite WG; Nicol HI. 1999. Field evaluation of the effects of the insect growth regulator tebufenozide on entomophagous arthropods and pests of apples. Australian Journal of Entomology 38(2):35-140.

Harris SA; Solomon KR. 1992. Human exposure to 2,4-D following controlled activities on recently sprayed turf. J. Environ. Sci. Health. B27(1): 9-22.

Hartung R. 1962. Ingestion of oil by waterfowl. Papers of the Michigan Academy of Science, Arts, and Letters. XLVIII: 49-55.

Hawkins D. 1992. RH-5992 Terrestrial Field Dissipation: Interim Report: Lab Project Number: 34-92-61: TR34-92-47: 34P-91-04. Unpublished study prepared by Rohm and Haas Co. MRID No.42931222. 128 pp.

Hawkins D. 1993. RH-5992: Terrestrial Field Dissipation at Two Sites in California: Lab Project Number: 34-93-59: 34P-91-04: TR34-93-59. Unpublished study prepared by Pan-Agricultural Labs, Inc. and Centre Analytical. MRID No.42991514. 675 pp.

Hawkins D. 1994. RH 5992: Terrestrial Field Dissipation in New York and Washington: Lab Project Number: 34/94/139. Unpublished study prepared by Pan-Agricultural Labs, Inc. and Centre Analytical Lab. MRID No.43797703. 741 pp.

Hawkins D. 1995. RH-5992: Environmental Fate in Canadian Forestry Environments: Lab Project Number: 34-95-128: TR34-95-128. Unpublished study prepared by Rohm and Haas Co. MRID No.43935603. 327 pp.

Hawkins D. 1996. Tebufenozide: Foliar Half-Life Estimation: Lab Project Number: 34-96-133. Unpublished study prepared by Rohm and Haas Co. MRID No.44200302. 9 pp.

Hawkins D. 1998. Tebufenozide: Inputs for Environmental Risk Assessment--Day 0 Residues on Fruit and Foliage and Foliar Half-Life: Lab Project Number: 34-98-133. Unpublished study prepared by Rohm and Haas Company. MRID No.44693202. 41 pp.

Hazelton G.; Quinn D. 1994. RH-5992 2F Agricultural Insect Growth Regulator: RH-5992 2F (ULV) Forestry Insect Growth Regulator: Occupational Risk Evaluation for Air-Blast and Aerial Application Methods: Lab Project Number: 94R/1. MRID No.43367002. 57 pp.

Hazelton G.; Quinn D. 1995a. Evaluation of Rat Reproduction Studies Conducted with RH-5992: Final Report: Lab Project Number: 95R-1068. Unpublished study prepared by Rohm and Haas Co. MRID No.43781707. 22 pp.

Hazelton G.; Quinn D. 1995b. RH-5992 Technical and CONFIRM 2F Agricultural Insecticide: Hazard Evaluation of RH-5992 Technical in Humans and Domestic Animals: Part I: Hazard Identification and Evaluation: Lab Project Number: 95R-. MRID No.43781708. 102 pp.

Hoberg J. 1992a. RH-5992 Technical--Toxicity to the Freshwater Green Alga, *Scenedesmus subspicatus*: Final Report: Lab Project Number: 91-11-4010: 90RC-0209: 86.0291.6130.460. Unpublished study prepared by Springborn. MRID No.42629501. 69 pp.

Hoberg, J. 1992b. RH-5992 Technical: Toxicity to the Freshwater Green Alga, *Selenastrum capricornutum*: Final Report: Lab Project Number: 91-11-4002: 90RC-0210: 86.0291.6131.430. Unpublished study prepared by Springborn. MRID No.42436245. 59 pp.

Hoberman A. 1991. RH-5992 Oral Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study in Rats: Lab Project Number: 018-016: 90RC-059: 90P-059. Unpublished study prepared by Argus Research Labs,. MRID No.42436225. 182 pp.

Hoerger F; Kenaga EE. 1972. Pesticide residues on plants: Correlation of representative data as a basis for estimation of their magnitude in the environment. In: Environmental Quality and Safety, Volume I: Global Aspects of Toxicology and Technology as Applied to the Environment. F. Coulston and F. Kerte (eds.). Academic Press, New York, NY. Pp. 9-28.

Hohing L. 1992a. Analytical Chemistry Report of Analyses to Determine RH-5992 Technical in Dietary Samples Administered in the 8-Day LC50 Bobwhite Quail Study. (Supp): Lab Project Number: 417-483: 87RC-054A. Unpublished study prepared by Hazleton Washington, Inc. 27 p. MRID 42436236.

Hohing L. 1992b. Analytical Chemistry Report of Analyses to Determine RH-5992 Technical in Dietary Samples Administered in the 8-Day LC50 Mallard Ducklings Study. (Supp): Lab Project Number: 417-483: 87RC-055A. Unpublished study prepared by Hazleton Washington, Inc. 27 p. MRID 42436238.

Holmes SB. 1998. Reproduction and nest behavior of Tennessee warblers *Vermivora peregrina* in forests treated with Lepidoptera-specific insecticides. J. Applied Ecology. 35: 185-194.

Hu W; Cook BJ; Ampasala DR; Zheng S; Caputo G; Krell PJ; Retnakaran A; Arif BM; Feng Q. 2004. Morphological and molecular effects of 20-hydroxyecdysone and its agonist tebufenozide on CF-203, a midgut-derived cell line from the spruce budworm, *Choristoneura fumiferana*. Arch Insect Biochem Physiol. 55(2):68-78.

ICRP (International Commission on Radiologic Protection). 1975. Report of the Task Group on Reference Man. Recommendations of the International Commission on Radiological Protection (ICRP) Publ. No. 23.   Pergamon Press, New York, NY.

Kaminski E. 1997. Ecological Hazard Evaluation and Risk Assessment of Tebufenozide and Confirm 70WSP Agricultural Insecticide for Cotton Application: Lab Project Number: 96R-1035. Unpublished study prepared by Rohm an. MRID No.44221903. 19 pp.   .

Keller L. 1994. RH-5992 Insecticide Use in Forestry in Canada: Ecological Hazard Evaluation and Assessment of Risk to Terrestrial Wildlife, Aquatic Organisms and Other Non-Target Species: Lab Project Number: 93R/1060. MRID No.43367001. 92 pp.

Keller L. 1996a. Specificity of Tebufenozide (RH-5992) Activity in Lepidoptera and Lack of Endocrine Effects in Nontarget Organisms: Summary of Laboratory and Field Studies: Lab Project Number: 96R-1071. Unpublished. MRID No.44125401. 19 pp.

Keller L. 1996b. Tebufenozide (RH-5992): Rapid Dissipation in Aquatic Matrices and Safety to Aquatic Nontarget Organisms in Forestry and Other Environments: Lab Project Number: 96R-1069: 34-95-144: 93RC-1003.   MRID No.44125402. 321 pp.

Keller L. 1997. A Comparison of the Effects of Diflubenzuron (Dimilin) and Tebufenozide (Confirm/Mimic) on Nontarget Terrestrial and Aquatic Arthropods and Aquatic Community Structure. Lab Project Number: 96R-1113. Unpublished study prepared by Rohm and Haas Co. 32 p. MRID 44221902

Keller L. 1998. Literature Summary for Tebufenozide (RH-5992). Rohm and Haas Report No. 98R-1055. Report submitted by John W. Long, Product Development Manager, Rohm and Hass, to David F. Thomas, USDA Forest Service, Dec. 1, 1999. 72 pp.

Keller L.; Brown B. 1998a. Mode of Action, Spectrum of Activity, Environmental Fate and Safety of Tebufenozide (RH-5992) to Nontarget Terrestrial and Aquatic Arthropods: Literature Summary: Lab Project Number: 98R-1055. Unpublished. MRID No.44693203. 70 pp.

Keller L.; Brown S. 1998b. Response to the United States Environmental Protection Agency's Reevaluation of Potential Ecological Risks Associated with Tebufenozide (RH-5992) Use on Pecans: Lab Project Number: 98R-1066. Unpublished. MRID No.44693201. 18 pp.

Kelly M. 1992. Product Chemistry Series 61: RH-5992 Technical Product Identity: Lab Project Number: APR/SH-92-212. Unpublished study prepared by Rohm & Haas Co. MRID No.42436201. 135 pp.

Knisel WG; Davis FM. 2000. GLEAMS (Groundwater Loading Effects of Agricultural Management Systems), Version 3.0, User Manual. U.S. Department of Agriculture, Agricultural Research Service, Southeast Watershed Research Laboratory, Tifton, GA. Pub. No.: SEWRL-WGK/FMD-050199. Report Dated May 1, 1999 and revised August 15, 2000. 194pp.

Kreutzweiser DP; Thomas DR. 1995. Effects of a new molt-inducing insecticide, tebufenozide, on zooplankton communities in lake enclosures. Ecotoxicology. 4:307-328.

Kreutzweiser DP; Capell SS; Wainio-Keizer KL; Eichenberg DC. 1994. Toxicity of a new molt-inducing insecticide (RH-5992) to aquatic macroinvertebrates. Ecotox. Environ. Safety. 28: 14-24.

Kreutzweiser DP; Gunn JM; Thompson DG; Pollard HG; Faber MJ. 1998. Zooplankton community responses to a novel forest insecticide, tebufenozide (Rh-5992), in littoral lake enclosures. Canadian Journal of Fisheries and Aquatic Sciences. 55(3): 639-648.

Laney RF; Hoffman RS. 1992. Methemoglobinemia secondary to automobile exhaust fumes. Am. J. Emerg. Med. 10(5): 426-428.

Legaspi J; Legaspi B C JR; Saldana RR. 1999. Laboratory and field evaluations of biorational insecticides against the Mexican rice borer (Lepidoptera: pyralidae) and a parasitoid (Hymenoptera: braconidae). Journal of Economic Entomology. 92(4): 804-810.

Liebhold A; McManus M. 1999. The evolving use of insecticides in gypsy moth management. Journal of Forestry. 97: 20-23.

Lo SC; Agar NC. 1986. NADH-methemoglobin reductase activity in the erythrocytes of newborn and adult mammals. Experientia. 42(11-12): 1264-1265.

Long J. 2000. RH-5992, 70W Residue Studies in Oranges: Lab Project Number: 34-99-06: 95225: 95225A. Unpublished study prepared by Rohm and Haas Company. 814 p. {OPPTS 860.1500}. MRID 45141009.

Martin RC; Hodder H; Sheppard G; Sharpe S. 1997. Determination by electrospray tandem mass spectrometry of the insecticide Mimic (Tebufenozide) following an aerial overspray of a small lentic pond. Analusis. 25 (7): M15-M19.

Mascarenhas VJ; Graves JB; Leonard BR; Burris E. 1998. Dosage-mortality responses of third instars of beet armyworm (Lepidoptera: noctuidae) to selected insecticides. Journal of Agricultural Entomology. 15(2): 125-140.

Mason RW; Johnson BL. 1987. Ergonomic factors in chemical hazard control. In: Handbook of Human Factors. Salveny, G; ed. John Wiley and Sons, New York, NY. Pp. 772-741.

McNamara P. 1991. RH-5992 Technical: The Chronic Toxicity to *Daphnia magna* under Flow-thru Conditions: Final Report: Lab Project Number: 91-7-3821: 86.0291.6128.130: 90RC-0207. Unpublished study prepared by Springborn. MRID No.42436243. 101 pp.

Medina P; Smagghe G; Budia F; Del Estal P; Tirry L; Vinuela E. 2002. Significance of penetration, excretion, and transovarial uptake to toxicity of three insect growth regulators in predatory lacewing adults. Arch Insect Biochem Physiol. 51(2):91-101

Medina P; Smagghe G; Budia F; Del Estal P; Tirry L; Vinuela E. 2003. Toxicity and Absorption of Azadirachtin, Diflubenzuron, Pyriproxyfen, and Tebufenozide after Topical Application in Predatory Larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environ. Entomol. 32(1): 196-203.

Moore JA. 1964. Physiology of the Amphibia. Academic Press, New York. 654 p. (Cited in USDA/APHIS 1993).

Morrison R; Carbone J; Parno J; et al. 1993. RH-75,992 Technical and RH-75,992 2F Formulation: Four-Week Dermal Toxicity Study in Rats: Final Report: Lab Project Number: 92P-150: 92R-150: SH-93-021. Unpublished study prepared by Rohm and Haas C. MRID No.42991507. 249 pp.

Moslen MT. 1996. Toxic Responses of the Liver. In: Casarett and Doull's Toxicology: The Basic Science of Poisons. 5th Edition. McGraw-Hill, Health Professions Division, New York, NY. Pp. 403-415.

Mulder P G JR; Prescott DJ. 1999a. Effect of insecticides on caterpillar and beneficial arthropod populations, 1998. Saxena, K. N. (Ed.). Arthropod Management Tests, Vol. 24. V+478p. Entomological Society of America: Lanham, Maryland, USA. 24(0): 268-269.

Mulder P G JR; Prescott DJ. 1999b. Effect of insecticides on potato leafhoppers, defoliating caterpillars, beneficial arthropods and peanut yield, 1998. Saxena, K. N. (Ed.). Arthropod Management Tests, Vol. 24. V+478p. Entomological Society of America: Lanham, Maryland, USA. 24(0): 269-270.

Nakagawa Y; Hattori K; Shimizu B-I; Akamatsu M; Miyagawa H; Ueno T. 1998. Quantitative structure-activity studies of insect growth regulators. XIV. Three-dimensional quantitative structure-activity relationship of ecdysone agonists including dibenzoylhydrazine analogs. Pesticide Science. 53(4): 267-277.

Nakamura A; Sagisaka A; Sakaguchi M; Suzuki K; Kuwano E. 1998. Precocious metamorphosis in *Bombyx mori* larvae and diapause termination in pharate first-instar larvae of *Antheraea yamamai* induced by 1-substituted imidazoles. Journal of Pesticide Science. 23(2): 117-122.

NRC (National Research Council). 1983. Risk assessment in the Federal government: managing the process. Washington, DC: National Academy Press; 176 p. + app.

Oberlander H; Silhacek DL; Leach CE 1998. Interactions of ecdysteroid and juvenoid agonists in *Plodia interpunctella* (Hubner). Arch. Insect Biochem. Physiol. 38 (2):91-99.

Osheroff M. 1991a. RH-5992: 13 Week Dietary Toxicity Study in Mice: Lab Project Number: 417-468: 89RC-102. Unpublished study prepared by Hazleton Labs America, Inc. MRID No.42436221. 456 pp.

Osheroff M. 1991b. RH-5992: 13-Week Dietary Toxicity Study in Rats: Final Report: Lab Project Number: 417-463: 89RC-101. Unpublished study prepared by Hazleton Washington, Inc. MRID No.42436219. 775 pp.

Palli SR, Primavara M, Tomkins W, Lambert D, and Retnakaran A. 1995. Age-specific effects of non-steroidal ecdysteriod agonist RH-5992 on te spruce budworm *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Eur. J. Entomol 92:325-332. Summarized in Keller and Brown 1998a.

Parno J. 1997. Acute Dermal Toxicity Study in Male and Female Rats: Mimic 240 LV Insecticide: Final Report: Lab Project Number: 96P-172: 96R-172. Unpublished study prepared by Rohm and Haas Company. MRID No.44727704. 20 pp.

Parno J; Gingrich S. 1994. Acute Dermal Toxicity Study in Male and Female Rats: Mimic 240 LV Insecticide: Final Report: Lab Project Number: 94P-162: 94R-162. Unpublished study prepared by Rohm and Haas Company. MRID No.44727703. 16 pp.

Parno J; Gingrich S. 1994. Acute Oral Toxicity Study in Male and Female Rats: Mimic 240 LV Insecticide: Final Report: Lab Project Number: 94P-165: 94R-165. Unpublished study prepared by Rohm and Haas Company. MRID No.44727702. 15 pp.

Patel C. 1998. Product Chemistry (End Use Product): Mimic 2 LV Insecticide: Lab Project Number: 98-204: APR-96-044: APR-94-196. Unpublished study prepared by Rohm and Haas Company. 164 p. MRID No.44727701.

Pauli BD; Coulson DR; Berrill M. 1999. Sensitivity of amphibian embryos and tadpoles to Mimic 240 LV insecticide following single or double exposures. Environ Toxicol Chem. 18(11): 2538-44.

Payne N; Retnakaran A; Cadogan B. 1997. Development and Evaluation of a Method for the Design of Spray Applications: Aerial Tebufenozide Applications to Control the Eastern Spruce Budworm, *Choristoneura fumiferana* (Clem.). Crop Prot. 16 (3):285-290.

Pietrantonio PV; Benedict JH. 1999. Effect of new cotton insecticide chemistries, tebufenozide, spinosad and chlorfenapyr, on *Orius insidious* and two *Cotesia* species. Southwestern Entomologist. 24(1): 21-29.

Quinn D; Hazelton G. 1997. Tebufenozide: Dietary Risk Assessment for Use of Confirm Agricultural Insecticide on a Variety of Crops: Lab Project Number: 97R-1054. Unpublished study prepared by Rohm and Haas Co. MRID No.44319101. 27 pp.

Quinn D. 1997. Toxicity Summary of the Tebufenozide Metabolites RH-120282, RH-120897, and RH-112703: Lab Project Number: 97R-1007: SBL 19-27: 94RC-152. Unpublished study prepared by Rohm and Haas Co. MRID No.44238701. 119 pp.

Reinert K. 1993a. RH-5992 Technical: Supplemental Report--One Generation Reproduction Study with the Bobwhite Quail (*Colinus virginianus*): Lab Project Number: 90RC-0267D: RH93-01: 90RC-267C. Unpublished study prepared. MRID No.42991502. 37 pp.

Reinert K. 1993b. Supplement to (RH-5992 Technical)--Toxicity to the Freshwater Green Alga, *Selenastrum capricornutum*: Lab Project Number: 90RC-0210A. Unpublished study prepared by Rohm and Haas Co. MRID No.42822201. 11 pp.

Reinert K. 1993c. RH-5992 Technical: Supplemental Report - One Generation Reproduction Study with the Bobwhite Quail. (*Colinus virgina* Nus). Project Number: 90RC/0267D, TD/93M/486, RH93/01. Unpublished study prepared by Rohm and Haas Co. 37p. MRID 46090102.

Reinert K. 1995a. RH-5992 Technical: Reproduction Study in Bobwhite Quail: Lab Project Number: 94RC-0023: RH59BWR-394: HWA 417-492. Unpublished study prepared by Ecological Planning and Toxicology, Inc. MRID No.43781701. 160 pp.

Reinert, K. 1995b. RH-5992 Avian Reproduction Toxicity: Supplement to Rohm and Haas Report No. 94RC-0023: Lab Project Number: 94RC-0023B. Unpublished study prepared by Rohm and Haas Co. MRID No.43781702. 7 pp.

Reinert K. 1995c. Supplement Statistical Analyses for RH-5992 Technical: Reproduction Study 94RC-0023 in Bobwhite Quail: Lab Project Number: 94RC-0023C: RH59BWR-394. Unpublished study. MRID No.43781703. 34 pp.

Reinert K; Robertson J; Dhadialla T. 1999. Full Life Cycle Toxicity of RH-5992 Technical to the Fathead Minnow (*Pimephales promelas*) Under Flow--Through Conditions: Lab Project Number: 94RC-0025A. Unpublished study prepared by Rohm and Haas. MRID No.44831501. 68 pp.

Retnakaran A, Hiruma K, Reddy Palli, and Riddiford LM. 1995. Molecular analysis of the mode of action of RH-5992, a Lepidopteran-specific, non-steroidal ecsysteroid agonist. Insect Biochem Molec Biol 25(1): 109-117. Summarized in Keller and Brown 1998a.

Retnakaran A; Macdonald A; Tomkins WL; Davis CN; Brownwright AJ; Palli SR. 1997a. Ultrastructural Effects of a non-steroidal ecdysone agonist, RH-5992, on the sixth instar of the Spruce budworm, *Choristoneura fumiferana*. J. Insect Physiol. 43(1): 55-68.

Retnakaran A; Smith LFR; Tomkins WL; Primavera MJ; Palli SR; Payne N; Jobin L 1997b. Effect of RH-5992, a nonsteroidal ecdysone agonist, on the Spruce budworm, *Toneura fumiferana* (Lepidoptera: Tortricidae): Laboratory, greenhouse and ground spray . Can. Entomol. 129(5): 871-885.

Rhodes J; Leak T. 1996. Full Life-Cycle Toxicity of RH-5992 Technical to the Fathead Minnow (*Pimephales promelas*) Under Flow-Through Conditions: Lab Project Number: 42408: 94RC-0025. MRID No.44221901. 1000 pp.

Richards J. 1992a. RH-5992: 52 Week Oral (Dietary Administration) Chronic Toxicity Study in the Beagle: Lab Project Number: 616/12: 7168-616/12: 90RC-206. Unpublished study prepared by Hazleton UK. MRID No.42931203. 394 pp.

Richards J. 1992b. RH-5992: 52 Week Oral (Dietary Administration) Chronic Toxicity Study in the Beagle: Report Supplement A, Photomicrographs: Lab Project Number: 616/12: 7168-616/12: 90RC-206A. MRID No.42931204. 12 pp.

Ruffle B; Burmaster DE; Anderson PD; Gordon HD. 1994. Lognormal distributions for fish consumption by the general U.S. population. Risk Anal. 14(4): 395-404.

Rumpf S; Hetzel F; Frampton C. 1997a. Lacewings (Neuroptera: hemerobiidae and chrysopidae) and integrated pest management: enzyme activity as biomarker of sublethal insecticide exposure. Journal of Economic Entomology. 90(1): 102-108.

Rumpf S; Frampton C; Chapman B. 1997b. Acute toxicity of insecticides to *Micromus tasmaniae* (Neuroptera: hemerobiidae) and *Chrysoperla carnea* (Neuroptera: chrysopidae): LC_{50} and LC_{90} estimates for various test durations. Journal of Economic Entomology. 90(6): 1493-1499.

Rumpf S; Frampton C; Dietrich DR 1998. Effects of conventional insecticides and insect growth regulators on fecundity and other able parameters of *Micromus tasmaniae* (Neuroptera: Hemerobiidae). J. Econ. Entomol. 91(1): 34-40.

Russell S; Martin R; Colbo M; Banoub J. 1996. The Effect of Aerial Overspray Using the Insecticide Mimic on Aquatic Invertebrates in a Small Lentic Pond. Rohm and Haas Report No. 96RC-1024. [Reproduced in Keller.1996b, pp. 171 ff].

Sames J; Elia M. 1993. RH-75,992 Technical: *Salmonella typhimurium* Gene Mutation Assay (Ames test): Lab Project Number: 93R-0094: 93P-094. Unpublished study prepared by Rohm and Haas Co. MRID No.42931210. 24 pp.

Sauphanor B; Bouvier J-C; Brosse V. 1999. Effect of an ecdysteroid agonist, tebufenozide, on the completion of diapause in susceptible and resistant strains of the codling moth, *Cydia pomonella*. Entomologia Experimentalis et Applicata. 90(2): 157-165.

SERA (Syracuse Environmental Research Associates, Inc.). 2002. Human Health and Ecological Risk Assessment Mimic (Tebufenozide) - Final Report, SERA TR 99-21-22-01e, dated May 26, 2000. Syracuse Environmental Research Associates, Inc., Fayetteville, NY. Available at http://www.fs.fed.us/foresthealth/pesticide/risk.htm.

SERA (Syracuse Environmental Research Associates, Inc.). 2001. Preparation of Environmental Documentation and Risk Assessments, SERA MD 2001-01a, draft dated July 2001. Syracuse Environmental Research Associates, Inc., Fayetteville, NY. Available at www.sera-inc.com.

SERA (Syracuse Environmental Research Associates, Inc.). 2004a. Documentation for the Use EXCEL Worksheets in Forest Service Risk Assessments (Version 3.01), SERA TD 2004-03.01a, dated March 13, 2004. Available at: www.sera-inc.com.

SERA (Syracuse Environmental Research Associates, Inc.). 2004b. Documentation for the Use of GLEAMS (Version 3) and Auxiliary Programs in Forest Service Risk Assessments (Version 2.04), SERA TD 2004-02.04a, dated February 8, 2004. Available at: www.sera-inc.com.

Sharma A; Schuck H. 1996. Hen Metabolism Study of RH-5992: Lab Project Number: 34-95-62. Unpublished study prepared by Rohm and Haas Co. and Analytical Biochemistry Labs. MRID No.44221906. 769 pp.

Sharma A. 1998. Storage Stability of RH-5992 and Metabolites in Forestry Matrices: Lab Project Number: 34-97-132: 34-96-10: 34P-94-54. Unpublished study prepared by Rohm and Haas Company and Centre Analytical Labs. 546 p. MRID 44727709.

Slama K. 1995. Hormonal status of RH-5849 and RH-5992 synthetic ecdysone agonists examined on several standard bioassays for ecdysteroids. Eur J Entomol 92: 317-323. Summarized in Keller and Brown 1998a.

Smagghe G; Degheele D 1994a. Action of a novel nonsteroidal ecdysteroid mimic, tebufenozide (RH-5992), on insects of different orders. Pestic. Sci. 42(2): 85-92.

Smagghe G; Degheele D 1994b. The significance of pharmacokinetics and metabolism to the biological activity of RH-5992 (tebufenozide) in *Spodoptera exempta*, *Spodoptera exigua* and *Leptinotarsa ineata*. Pestic. Biochem. Physiol. 49(3): 224-234.

Smagghe G and Degheele S. 1995. Selectivity of nonsteroidal ecdysteroid agonists RH-5849 and RH-5992 to Nypmhs and adults of predatory soldier bugs *Podisus nigrispinus* and *P. Maculiventris* (Hemiptera: Pentaonidae). J Econ Entomol 88(1):40-45. Summarized in Keller and Brown 1998a.

Smagghe G; Degheele D 1997. Comparative toxicity and tolerance for the ecdysteroid mimic tebufenozide in a laboratory and field strain of Cotton leafworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 90(2): 278-282.

Smagghe G; Bohm GA; Richter K; Degheele D 1995. Effect of nonsteroidal ecdysteroid agonists on ecdysteroid titer in *Spodoptera exigua* and *Otarsa decemlineata*. J. Insect Physiol. 41(11): 971-974.

Smagaghe G; Eelen H; Verschelde E; Richter K; Degheele D 1996a. Differential effects of nonsteroidal ecdysteroid agonists in coleoptera and lepidoptera: Analysis of evagination and receptor binding in imaginal discs. Insect. Biochem. Mol. Biol. 26(7): 687-695.

Smagghe G; Vinuela E; Budia F; Degheele D 1996b. *In vivo* and *in vitro* effects of the nonsteroidal ecdysteroid agonist tebufenozide on cuticle ion in *Spodoptera exigua*: An ultrastructural approach. Arch. Insect Biochem. Physiol. 33(2): 121-124.

Smagghe G; Vinuela E; Budia F; Degheele D 1997. Effects of the non-steroidal ecdysteroid mimic tebufenozide on the Tomato looper *Deixis chalcites* (Lepidoptera: Noctuidae): An ultrastructural analysis. Arch. Insect Biochem. Physiol. 35: 179-190.

Smagghe G; Dhadialla TS; Derycke S; Tirry L; Degheele D. 1998. Action of the ecdysteroid agonist tebufenozide in susceptible and artificially selected beet armyworm. Pesticide Science. 54(1): 27-34.

Smagghe G; Gelman D; Tirry L. 1999a. In vivo and in vitro effects of tebufenozide and 20-hydroxyecdysone on chitin synthesis. Archives of Insect Biochemistry and Physiology. 41(1): 33-41.

Smagghe G; Nakagawa Y; Carton B; Mourad A; Fujita T; Tirry L. 1999b. Comparative ecdysteroid action of ring-substituted dibenzoylhydrazines in *Spodoptera exigua*. Archives of Insect Biochemistry and Physiology. 41(1): 42-53.

Smith RP. 1996. Toxic Responses of the Blood. In: Casarett and Doull's Toxicology: The Basic Science of Poisons. 5th Edition. McGraw-Hill, Health Professions Division, New York, NY. Pp. 335-353.

Song MY; Brown JJ. 1998. Osmotic effects as a factor modifying insecticide toxicity on *Aedes* and *Artemia*. Ecotoxicology and Environmental Safety. 41(2): 195-202.

Song MY; Stark JD; Brown JJ 1997. Comparative toxicity of four insecticides, including imidacloprid and tebufenozide, to aquatic arthropods. Environ. Toxicol. Chem. 16(12): 2494-2500.

SRC (Syracuse Research Corporation). 1999. Logkow Program. SRC's LogKow (KowWin) Program. esc-plaza.syrres.com/interkow/logkow.htm.

Struble C; Hazelton G. 1992. (Carbon 14)-RH-5992: Pharmacokinetic Study in Rats: Final Report: Lab Project Number: HWI 6228-108: 91P-004: 91RC-004. Unpublished study prepared by Hazleton Wisconsin, Inc. MRID No.42931211. 257 pp.

Suckling DM; Walker J TS; Wearing CH. 1999. Ecological impact of three pest management systems in New Zealand apple orchards. Agriculture Ecosystems and Environment. 73(2): 129-140.

Sun X; Barrett BA. 1999. Fecundity and fertility changes in adult codling moth (Lepidoptera: tortricidae) exposed to surfaces treated with tebufenozide and methoxyfenozide. Journal of Economic Entomology. 92(5): 1039-1044.

Sundaram KMS. 1994a. Degradation kinetics of tebufenozide in model aquatic systems under controlled laboratory conditions. J. Environ. Sci. Health, Part B, Pestic. Food Contam. Agric. Wastes. (6):1081-1104.

Sundaram KMS. 1994b. Rain-washing of Mimic, RH-5992, from balsam fir foliage following application of two formulations. J. Environ. Sci. Health, Part B, Pestic. Food Contam. Agric. Wastes. 29(3):541-579.

Sundaram KMS. 1995. Photostability and rainfastness of tebufenozide deposits on fir foliage. 207th Ann. Meeting Amer. Chem. Soc. 134-152.

Sundaram KMS. 1996. Leaching, mobility and persistence of tebufenozide in columns packed with forest litter II. J. Environ. Sci. Health. B31(6): 1215-1239.

Sundaram KMS. 1997a. Persistence and mobility of tebufenozide in forest litter and soil ecosystems under field laboratory conditions. Pestic. Sci. 51(2): 115-130. .

Sundaram KMS. 1997b. Uptake, elimination and biochemical effects of azadirachtin and tebufenozide in algae. J. Environ. Sci. Health. B31(2): 295-312.

Sundaram KMS; Nott R; Curry J 1996a. Deposition, persistence and fate of tebufenozide (RH-5992) in some terrestrial and aquatic components of a boreal forest environment after aerial application of Mimic. J. Environ. Sci. Health. B31(4): 699-750.

Sundaram KMS; Sundaram A; Sloane L 1996b. Foliar persistence and residual activity of tebufenozide against Spruce budworm larvae. Pestic. Sci. 47(1): 31-40.

Sundaram A; Sundaram KMS; Sloane L 1997a. Effect of physical properties and surfactant concentrations on phase separation in tebufenozide droplets: Persistence and bioactivity of the insecticide in spruce foliage. J. Environ. Sci. Health. B32(2): 235-260.

Sundaram A; Sundaram KMS; Sloane L; Nott R; Curry J. 1997b. Influence of additives in the end-use mixes of tebufenozide on deposition, adhesion and persistence in spruce foliage. J. Environ. Sci. Health. B32(4): 497-522.

Surprenant D. 1994. Supplement to: RH-5992 Technical--Toxicity to Fathead Minnow (*Pimephales promelas*) Embryos and Larvae: Lab Project Number: 91-9-3923: 86.0291.6133. 120: 90RC-212B. Unpublished study prepared by Spring. 6 pp. MRID No.43145701.

Swenson R; Solomon H. 1992. RH-5992:Oral (Gavage) Developmental Toxicity Study in Rabbits: Lab Project Number: 90P-201: 90R-201. Unpublished study prepared by Rohm & Haas Co. MRID No.42436227. 241 pp.

Swenson R; Gillette D; Parno J. 1994. RH-75,992: Acute Oral (Gavage) Neurotoxicity Study in Rats: Final Report: Lab Project Number: 93P-093: 93R-093. Unpublished study prepared by Rohm and Haas Co. MRID No.43781706. 1801 pp.

Thilagar A. 1988. Test for Chemical Induction of Chromosome Aberration Using Monolayer Cultures of Chinese Hamster Ovary (CHO) Cells with and without Metabolic Activation: Lab Project Number: 0067-3100: 88RC-011. Unpublished study. MRID No.42459803. 105 pp.

Thilagar A. 1990a. RH-5992: Test for Chemical Induction of Gene Mutation at the HGPRT Locus in Cultured Chinese Hamster Ovary (CHO) Cells with and without Metabolic Activation: Final Report: Lab Project Number: 0111-250. Unpublished study prepared by Sitek Research Labs. MRID No.42436230. 43 pp.

Thilagar A. 1990b. Test for Chemical Induction of Unscheduled DNA in Rat Primary Hepatocytes Cultures by Autoradiography: Lab Project Number: 0111-5100: 89RC-098. Unpublished study prepared by Sitek Research Labs. MRID No.42436232. 80 pp.

Tillman A. 2000. Confirm Agricultural Insecticide. (Tebufenozide): Justification for Removal of Grazing Restriction from Pome Fruit and Tree Nut Use Directions: Lab Project Number: AMT 00-079. Unpublished study prepared by Rohm and Haas Co. 31 p. MRID 45209401.

Trutter J. 1992a. RH-5992: 18-Month Dietary Oncogenicity Study in Mice: Final Report: Lab Project Number: 417-473: 90RC-061. Unpublished study prepared by Hazleton Washington, Inc. MRID No.42931205. 2754 pp.

Trutter J. 1992b. RH-5992: 18-Month Dietary Oncogenicity Study in Mice: Report Supplement A, Photomicrographs: Lab Project Number: 417-473: 90RC-061A. Unpublished study prepared by Hazleton Washington, Inc. MRID No.42931206. 122 pp.

Trutter J. 1992c. RH-5992: 24-Month Combined Dietary Chronic Toxicity and Oncogenicity Study in Rats: Final Report: Lab Project Number: 417-472: 90RC-060. Unpublished study prepared by Hazleton Washington, Inc. MRID No.42931208. 4188 pp.

U.S. EPA (U.S. Environmental Protection Agency). 1984. Users Manual for the Pesticide Root Zone Model (PRZM), Release 1, EPA-600/3-84-109.

U.S. EPA (U.S. Environmental Protection Agency). 1985. Development of Statistical Distributions or Ranges of Standard Factors Used in Exposure Assessments, Report prepared by GCA Corp., Chapel Hill. Available from NTIS: PB85-242667.

U.S. EPA (U.S. Environmental Protection Agency). 1989. Recommendations for and Documentation of Biological Values for use in Risk Assessment. U.S. EPA, Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH. ECAO-CIN-554. [pagination not continuous].

U.S. EPA (U.S. Environmental Protection Agency). 1992. Dermal Exposure Assessment: Principles and Applications. EPA/600/8-91/011B. Interim Report. Exposure Assessment Group, Office of Health and Environmental Assessment, Washington, DC.

U.S. EPA (U.S. Environmental Protection Agency). 1993. Wildlife Exposure Factors Handbook. Volumes 1 and 2. EPA/600/R-93/187a,b. Pagination not continuous. Available NTIS: PB94-174778 and PB94-174779.

U.S. EPA (U.S. Environmental Protection Agency). 1996. Exposure Factors Handbook. Office of Research and Development, National Center for Environmental Assessment, U.S. EPA, Washington, DC. EPA/600/P-95/002Ba-c. Avail. NTIS: PB97-117683, 97-117691, PB97-117709.

U.S. EPA (U.S. Environmental Protection Agency). 1999a. Tebufenozide Pesticide Tolerances for Emergency Exemptions. Federal Register. 64(51): 13088-13094.

U.S. EPA (U.S. Environmental Protection Agency). 1999b. Tebufenozide Pesticide Tolerance Final Rule. Federal Register. 64(183): 51251-51258.

U.S. EPA (U.S. Environmental Protection Agency). 1999c. Tebufenozide: Tier I Drinking Water EECs for use in the Human Health Risk Assessment. Memo from Dana Spatz and Jim Cowles dated January 19, 1999. (Copy courtesy of Janet Bressant, Public Information and Records Integrity Branch, U.S. EPA/OPP).

U.S. EPA (U.S. Environmental Protection Agency). 1999d. EFED risk assessment for Section 3 registration of tebufenozide (Confirm 2F and 70WSP) for use on tree nuts and almond hull; DP Barcode: D255779. Memo from N.E. Federoff, M. Rexrode, and J. Cowles dated July 13, 1999. (Copy courtesy of Janet Bressant, Public Information and Records Integrity Branch, U.S. EPA/OPP).

U.S. EPA (U.S. Environmental Protection Agency). 1999e. EFED risk assessment for Section 3 registration of tebufenozide (Confirm 2F and 70WSP). Memo from M. Rexrode, and J. Cowles dated March 1, 1999. (Copy courtesy of Janet Bressant, Public Information and Records Integrity Branch, U.S. EPA/OPP).

U.S. EPA (United States Environmental Protection Agency). 2004. IRIS Database. On-Line Search as of March 7, 2004. Address: http://www.epa.gov/iriswebp/iris/ index.html.

USDA/APHIS. 1993. Nontarget Risk Assessment for the MEDFLY Cooperative Eradication Program. USDA Animal and Plant Health Inspection Service. February 1993.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1989a. Final Environmental Impact Statement: Vegetation Management in the Coastal Plain/Piedmont, Management Bulletin R8-MB-23, dated January, 1989. 1213 pp.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1989b. Draft Environmental Impact Statement: Vegetation Management in the Ozark/Ouachita Mountains, Management Bulletin R8-MB-23, dated June, 1989. 499 pp.

USDA/FS (U.S. Department of Agriculture/Forest Service). 1989c. Final Environmental Impact Statement: Vegetation Management in the Appalachian Mountains, Management Bulletin R8-MB-38, dated July, 1989. 1104 pp.

USDA (U.S. Department of Agriculture). 1995. Gypsy Moth Management in the United States: A Cooperative Approach. Final Environmental Impact Statement. Appendix F (Human Health Risk Assessment) and Appendix G (Ecological Risk Assessment).

USGS (U.S. Geological Survey). 1998. Data on Pesticides in Surface and Ground Water of the United States., Results of the National Water Quality Assessment Program (NAWQA). Revised Oct. 23, 1998. http://water.wr.usgs.gov/pnsp/use92/tebufzd.html .

U.S. Weather Service. 1998. NWS Climate Tables, 1961-1990. http://www.nws.noaa.gov/climatex.shtml. Files downloaded on January 14, 1999.

van Hemmen JJ. 1992. Agricultural pesticide exposure data bases for risk assessment. Rev. Environ. Contam. Toxicol. 126: 1-85.

Valentine BJ; Gurr GM; Thwaite WG 1996. Efficacy of the Insect Growth Regulators Tebufenozide and Fenoxycarb for Lepidopteran Pest Control in Apples and Their Compatibility with Biological Control for Integrated Pest Management. Aust. J. Exp. Agric. 36 (4):501-506.

van der Kolk J. 1997. RH-5992: Chronic Effects on Midge Larvae (Chironomus riparius) in a Water/Sediment System: Final Report: Lab Project Number: 95RC-0196: 96-047-1007: 1007.011.173. Unpublished study prepared by Spring. MRID No.44198301. 109 pp.

van de Vrire M, Smagghe G, and Degheele D. 1996. Laboratory test method to evaluate the effect of 31 pesticides on the predatory bug Orius laevegatus (Het: Anthocoridae). Entomolphaga 41(2):235-243. Summarized in Keller and Brown 1998a.

Walgenbach JF. 1995. Effect of various insecticide programs on pest and beneficial arthropods in apples. Rohm and Hass Cooperator Report No. 2599521. Unpublished report summarized in Keller 1998.

Webb RE; Peiffer R; Fuester RW; Thorpe KW; Calabrese L; Mclaughlin JM. 1998. An evaluation of the residual activity of traditional, safe, and biological insecticides against the gypsy moth. Journal of Arboriculture. 24(5): 286-292.

Wederbrand K; Potter D. 1993. Carbon 14-RH-5992: Dermal Absorption in Male Rats: Final Report: Lab Project Number: 92R-183: 92P-183. Unpublished study prepared by Rohm and Haas Co. MRID No.42991510. 61 pp.

West RJ; Thompson D; Sundaram KMS; Sundaram A; Retnakaran A; Mickle R 1997. Efficacy of aerial applications of *Bacillus thuringiensis* berliner and tebufenozide against Stern hemlock looper (Lepidoptera: Geometridae). Can. Entomol. 129(4): 613-626.

Whiting DC; Jamieson LE; Connolly PG. 1999. Effect of sublethal tebufenozide applications on the mortality responses of *Epiphyas postvittana* (Lepidoptera: tortricidae) larvae exposed to a high-temperature controlled atmosphere. Journal of Economic Entomology. 92(2): 445-452.

Wirth S; Vogel K. 1988. Cow's milk protein intolerance in infants with methemoglobinemia and diarrhea. Eur. J. Ped. (Berlin). 148(2): 172.

Woebkenberg NR; Mostardi RA; Ely DL; Worstell D. 1981. Carboxyhemoglobin and methemoglobin levels in residents living in industrial and nonindustrial communities. Environ. Res. 26(2): 347-352.

TEBUFENOZIDE

ESTIMATED ANNUAL AGRICULTURAL USE

Average use of
Active Ingredient
Pounds per square mile
of county per year

☐ No Estimated Use
☐ < 0.027
🟩 0.027 – 0.089
🟨 0.090 – 0.204
🟧 0.205 – 0.458
🟥 >= 0.459

Crops	Total Pounds Applied	Percent National Use
cotton	42, 104	100. 00

Figure 2-1: Agricultural Use of Tebufenozide on Cotton in 1992 (USGS 1998).

Table 2-1. Selected physical and chemical properties of tebufenozide with selected additional properties for the commercial formulation Mimic.

Appearance, ambient	Mimic: off-white, cream color liquid. (C&P Press 2004)
	Tebufenozide, technical: while solid (Kelly 1992)
Bioconcentration factor	151 in whole fish (Dong and Hawkins. 1993)
	16 in edible tissue (Dong and Hawkins. 1993)
CAS number	112410-23-8 (C&P Press 2004; Kelly 1992)
Commercial formulations	Mimic 2LV; Confirm 2F
EPA Registration Number	707-237 (Patel 1998)
Foliar half-time (days)	2.8 to 13.3 days (Hawkins 1998)
	11.3 to 14 days (Kaminski 1997)
	about 18.4 to 58.7 days (Sundaram et al. 1996a, Table 6, p. 725)
	about 20 days (white spruce) (Sundaram et al. 1996b,)
Foliar wash-off fraction	0.3 to 0.7 Sundaram et al. (1997b, Table 6, p. 514)
	0.2 to 0.8 Sundaram (1994b)
log $K_{o/w}$	4.25 (Hawkins 1995)
	4.25 (SRC 1999)[$K_{o/w}$ = 17,800]
Molecular weight	352.48 (Patel 1998)
pH	6.5-7.5 (C&P Press 2004)
Photolysis (days)	98[soil surface] (Hawkins 1995)
	67[in aqueous solution] (Hawkins 1995)
Soil half-time (days)	99 to 101[aerobic] (Hawkins 1995)
	66[aerobic] (Kaminski 1997)
Soil sorption, $K_{o/c}$	572 (Hawkins 1995)
Specific Gravity	Mimic: 1.0 (C&P Press 2004)
Synonyms	3,5-dimethyl-, 1-(1,1-dimethylethyl)-benzoic acid (C&P Press 2004)
	N-tert-butyl-N'-(4-ethylbenzoyl)-3,5-dimethylbenzol)hydrazide (Kaminski 1997)
	RH-5992 (Kelly 1992), Confirm
Vapor pressure	17 mm Hg @ 20°C/68°F (C&P Press 2004)
	2×10^{-8} torr at 25°C (Kaminski 1997)
Volatility	60% (C&P Press 2004)
Water solubility (mg/L)	0.83 (Kaminski 1997)

Table 3-1: Chemical and site parameters used in GLEAMS modeling for tebufenozide.

Chemical Specific Parameters

Parameter	Clay	Loam	Sand	Comment/ Reference
Halftimes (days)				
Aquatic Sediment		179		U.S. EPA 1999e, p. 5
Foliar		13.4		Note 1
Soil	100	270	730	Note 2
Water		67		Note 3
Ko/c, mL/g		572		Note 4
K_d, mL/g	7.8	4.4	1.7	Note 5
Water Solubility, mg/L		0.83		Kaminski 1997
Foliar wash-off fraction		0.5		Note 6
Fraction applied to foliage		0.8		

Note 1   Geometric mean of range of values from Table 2-1: 3 to 60 days.

Note 2   The soil half time for sand is taken as 730 days, the value used by U.S. EPA (1999e) in PRZM/EXAMS modeling. For clay, a soil halftime of 100 days is used (Hawkins 1995). As an intermediate value, the geometric mean of this range is used for loam.

Note 3   Photolysis halftime used by U.S. EPA 1999e from study by Hawkins 1995.

Note 4   This is taken from Hawkins (1995) and is identical to the value used by U.S. EPA (1999e) in the PRZM/EXAMS modeling

Note 5   Taken from U.S. EPA (1999e), Table 1, p. 6.

Note 6   Sundaram et al. (1997) have reported wash-off fractions 30% to 70% (Table 6, p. 514). Somewhat wider ranges, 20% to 80%, have been reported by Sundaram (1994b). For the GLEAMS modeling, a central value of 50% is used.

Site Parameters
(see SERA 2004b for details)

Pond   1 hectare pond, 2 meters deep, with a 0.01 sediment fraction. 10 hectare square field (1093' by 1093') with a root zone of 12 inches.

Stream   Base flow rate of 710,000 L/day with a flow velocity of 0.08 m/second or 6912 meters/day. Stream width of 2 meters (about 6.6 feet'). 10 hectare square field (1093' by 1093') with a root zone of 12 inches.

Table 3-2: Summary of modeled concentrations of tebufenozide in streams (all units are µg/L or ppb)

Annual Rainfall (inches)	Rainfall per Event (inches)[1]	Clay Average	Clay Maximum	Loam Average	Loam Maximum	Sand Average	Sand Maximum
		Concentration per lb/acre applied (from GLEAMS)					
5	0.14	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
10	0.28	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
15	0.42	0.69713	19.95600	0.00878	0.29002	1.90923	52.54274
20	0.56	1.68973	54.33504	0.06773	1.43491	5.30526	101.05556
25	0.69	2.55255	91.00476	0.16814	3.12871	7.05234	111.28758
50	1.39	4.09339	219.00699	0.77041	11.44738	6.85127	93.61309
100	2.78	3.52070	317.12471	1.34698	30.36614	4.42689	88.43373
150	4.17	2.70849	334.75298	1.35142	45.96028	3.16969	88.64864
200	5.56	2.16187	320.13751	1.24326	55.46092	2.43988	87.51616
250	6.94	1.78771	287.69153	1.12607	60.75455	1.97609	84.88519
Application rate:		**0.12**	lbs/acre				
		Concentration at above application rate					
5	0.14	0	0	0	0	0	0
10	0.28	0	0	0	0	0	0
15	0.42	0.083656	2.39472	0.00105	0.034802	0.2291076	6.3051288
20	0.56	0.2027676	6.5202048	0.00813	0.1721892	0.6366312	12.126667
25	0.69	0.306306	10.920571	0.020177	0.3754452	0.8462808	13.35451
50	1.39	0.4912068	26.280839	0.092449	1.3736856	0.8221524	11.233571
100	2.78	0.422484	38.054965	0.1616376	3.6439368	0.5312268	10.612048
150	4.17	0.3250188	40.170358	0.1621704	5.5152336	0.3803628	10.637837
200	5.56	0.2594244	38.416501	0.1491912	6.6553104	0.2927856	10.501939
250	6.94	0.2145252	34.522984	0.1351284	7.290546	0.2371308	10.186223

[1] Rain is assumed to occur at the same rate every 10th day – i.e., 36 rainfall events per year.

Table 3-3: Summary of modeled concentrations of tebufenozide in ponds (all units are µg/L or ppb)

Annual Rainfall (inches)	Rainfall per Event (inches)[1]	Clay		Loam		Sand	
		Average	Maximum	Average	Maximum	Average	Maximum
		Concentration per lb/acre applied (from GLEAMS)					
5	0.14	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
10	0.28	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
15	0.42	1.62583	3.41905	0.01831	0.04465	4.17974	8.26554
20	0.56	3.01439	9.47016	0.10599	0.18515	8.82060	13.48834
25	0.69	4.18885	16.64130	0.23102	0.36543	10.95654	15.44082
50	1.39	7.25113	51.67100	0.93903	1.28274	11.29006	26.68412
100	2.78	8.47509	103.59184	2.06369	6.79246	8.75309	39.33410
150	4.17	7.95210	134.03042	2.47999	16.52847	7.16252	45.03134
200	5.56	7.23386	157.87981	2.59791	25.60810	6.09099	47.50864
250	6.94	6.58435	168.88316	2.59975	32.69145	5.32904	48.43668
Application rate:		**0.12**	lbs/acre				
		Concentration at above application rate					
5	0.14	0	0	0	0	0	0
10	0.28	0	0	0	0	0	0
15	0.42	0.1950996	0.410286	0.0022	0.00536	0.5015688	0.9918648
20	0.56	0.3617268	1.1364192	0.012719	0.022218	1.058472	1.6186008
25	0.69	0.502662	1.996956	0.027722	0.043852	1.3147848	1.8528984
50	1.39	0.8701356	6.20052	0.1126836	0.1539288	1.3548072	3.2020944
100	2.78	1.0170108	12.431021	0.2476428	0.8150952	1.0503708	4.720092
150	4.17	0.954252	16.08365	0.2975988	1.9834164	0.8595024	5.4037608
200	5.56	0.8680632	18.945577	0.3117492	3.072972	0.7309188	5.7010368
250	6.94	0.790122	20.265979	0.31197	3.922974	0.6394848	5.8124016

[1] Rain is assumed to occur at the same rate every 10th day – i.e., 36 rainfall events per year.

Table 3-4: Estimated Environmental Concentrations (µg/L or ppb) of tebufenozide in surface and groundwater at two applications of 0.12 lb a.i./acre (0.134 kg/ha), three days apart.

Scenario	Peak	Long-Term Average
MODELING FOR THIS RISK ASSESSMENT		
Direct Spray of Pond (Worksheet 04b)	6.73	N/A
Pond, drift at 100 feet (Worksheet 04b)	0.13	N/A
GLEAMS, Stream	10 (0.03 to 40)	0.3 (0.001 to 0.8)
GLEAMS, Pond	5 (0.005 to 20)	0.5 (0.002 to 1.4)
GENEEC Version 2, Pond	8.21	1.5 [90 day value of 6.01 x 90/360]
Sci-Grow 2.3, groundwater	0.093	
OTHER MODELING		
U.S. EPA/OPP 1999e.PRZM/EXAMS modeling of application to apples, Pond	8.7 ppb at 6x0.31 lb/ac	5.4 ppb at 6x0.31 lb/ac
U.S. EPA/OPP 1999e.PRZM/EXAMS modeling of application to cotton, Pond	17 ppb at 4x0.25 lb/ac	8.2 ppb at 4x0.25 lb/ac
MONITORING STUDIES		
Sundarum et al. 1996a	At an application rate of 2x0.070 kg/ha (0.062 lb/acre) with a 4 day interval. Peak stream concentrations of 1.32 ppb and peak pond concentrations of 5.31 ppb. Concentrations were below the limit of quantization limit of 0.04 µg/L by day 24 after application. Pond=300,000 liters in volume, 500 m^2 surface area, 0.6 m deep. Stream width=2m, depth=20 cm, 7 m/min flow.	

Table 3-5: Concentrations of tebufenozide in surface water used in this risk assessment (see Section 3.2.3.4.6 for discussion).

At application rate:	0.12	lb/acre, 2 applications, 3 days apart	
		Peak Concentration (ppb or µg/L)	Longer Term Concentration (ppb or µg/L)
	Central	10	0.5
	Lower	0.005	0.002
	Upper	40	1.4
Water contamination rate [1]		**mg/L per lb/acre applied, 2 applications, 3 days apart**	
		Peak Concentration (mg/L per lb/acre)	Longer Term Concentration (mg/L per lb/acre)
	Central	8.33e-02	4.17e-03
	Lower	4.17e-05	1.67e-05
	Upper	3.33e-01	1.17e-02

[1] Water contamination rates – concentrations in units of mg/L expected at an application rate of 1 lb/acre. These values are entered into Worksheet B06a for diflubenzuron. This rate is adjusted to the program application rate in all worksheets involving exposure to contaminated water.

Table 4-1: Summary of modeled concentrations of tebufenozide in soil (all units are mg/kg or ppm), two applications spaced three days apart.

Annual Rainfall (inches)	Rainfall per Event (inches)[1]	Clay		Loam		Sand	
		Average	Maximum	Average	Maximum	Average	Maximum
		Concentration per lb/acre applied (from GLEAMS)					
5	0.14	0.14894	0.33141	0.29680	0.49427	0.50678	0.84666
10	0.28	0.15592	0.33655	0.31226	0.51438	0.51705	0.86709
15	0.42	0.14905	0.33070	0.29440	0.48949	0.48422	0.79343
20	0.56	0.14349	0.32703	0.29249	0.48803	0.43053	0.67757
25	0.69	0.13746	0.32353	0.29102	0.48656	0.37176	0.57116
50	1.39	0.10849	0.30803	0.27746	0.46370	0.20593	0.34765
100	2.78	0.06705	0.27677	0.22935	0.39646	0.10536	0.28079
150	4.17	0.04360	0.24522	0.19143	0.35247	0.07083	0.27603
200	5.56	0.03094	0.21427	0.16493	0.32387	0.05381	0.27361
250	6.94	0.02313	0.18274	0.14567	0.30341	0.04358	0.27084
Application rate:		**0.12**	lbs/acre				
		Concentration at above application rate					
5	0.14	0.017873	0.039769	0.035616	0.059312	0.060814	0.1015992
10	0.28	0.01871	0.040386	0.037471	0.061726	0.062046	0.1040508
15	0.42	0.017886	0.039684	0.035328	0.058739	0.058106	0.095212
20	0.56	0.017219	0.039244	0.035099	0.058564	0.051664	0.081308
25	0.69	0.016495	0.038824	0.034922	0.058387	0.044611	0.068539
50	1.39	0.013019	0.036964	0.033295	0.055644	0.024712	0.041718
100	2.78	0.00805	0.033212	0.027522	0.047575	0.012643	0.033695
150	4.17	0.00523	0.029426	0.022972	0.042296	0.0085	0.033124
200	5.56	0.00371	0.025712	0.019792	0.038864	0.00646	0.032833
250	6.94	0.00278	0.021929	0.01748	0.036409	0.00523	0.032501

[1] Rain is assumed to occur at the same rate every 10th day – i.e., 36 rainfall events per year.

Table 4-2: Summary of tebufenozide toxicity values used in ecological risk assessment

Organism	Endpoint	Toxicity Value	Reference, Species
Mammals (Rats and Rabbits)	Acute NOAEL, reproduction	1000 mg/kg	Swenson and Solomon 1992 (rabbits) Hoberman 1991 (rats)
	Chronic NOAEL, toxicity	1.8 mg/kg/day	Richards 1992a
Birds (Bobwhite Quail)	Acute NOAEL	2150 mg/kg	Fletcher 1987
	Chronic NOAEL	15 mg/kg/day	Beavers et al. 1993b [1]
Terrestrial Invertebrates			
Honey bee	NOEC	2500 mg/kg	Atkins (1990) and Chan (1995)
Tolerant Insect Species	NOEC	0.24 lb a..i. /acre	Mulder and Prescott 1999a,b
Sensitive Lepidoptera	LOEC	0.03 lb a..i./acre	Butler et al. (1997)
Earthworm	NOEC	1000 mg/kg soil	Garvey (1992)
Fish Acute			
Sensitive (Bluegills)	NOEC	0.39 mg/L	Graves and Smith (1992b)
Tolerant (Trout)	NOEC	1.9 mg/L	Graves and Smith (1992c)
Fish Chronic			
Sensitive/Tolerant (Fathead Minnows)	LOEC, reproduction	0.048 mg/L	Rhodes and Leak (1996) as interpreted by U.S. EPA (1999e) [3]
Aquatic Invertebrates, Acute			
Sensitive (Midge larvae)	NOEC	0.12 mg/L	van der Kolk (1997)
Tolerant (Daphnids)	NOEC	0.82 mg/L	Graves and Smith (1992a)
Aquatic Invertebrates, Chronic			
Sensitive (Midge larvae)	NOEC, reproduction	0.0035 mg/L	van der Kolk (1997)
Tolerant (Daphnids)	NOEC, reproduction	0.029 mg/L	McNamara (1991)
Aquatic Plants			
Sensitive (*Scenedesmus subspicatus*)	NOEC for growth	0.077 mg/L	Hoberg (1992a)
Tolerant (*Selenastrum capricornutum*)	NOEC for growth	0.64 mg/L	Reinert (1993b)

[1] Other studies are available indicating higher NOAELs. See 4.3.2.2 for discussion.
[2] Other studies are available indicating no effects on tolerant invertebrates at application rates up to 0.25 lb/acre.
    See Table 4-3 and Section 4.3.2.3 for discussion.
[3] See Section 4.3.3.1 for a discussion of interpretation of studies.

Table 4-3: Summary of field studies on the effects of tebufenozide on terrestrial invertebrates [1]

Range of Application Rates (lb a.i./acre)	Species	
	No Adverse Effects	**Adverse Effects**
0.03 - <0.06	abundance of non-target arthropods other than macrolepidoptera (0.03 – Butler et al. 1997)	abundance of various macrolepidoptera (0.03 – Butler et al. 1997)
0.06 - < 0.12	abundance of non-target arthropods other than macrolepidoptera (0.06 – Butler et al. 1997)	abundance of various macrolepidoptera (0.06 – Butler et al. 1997) spruce budworm (0.06 – Cadogan et al. 1997)
0.12 - < 0.24	spiders, lacewings, and predatory mites (0.23 – Gurr et al. 1999) Mexican rice borer (0.12 and 0.18 – Legaspi et al. 1999) various beneficial arthropods* (0.125 – Mulder and Prescott 1999a)	spruce budworm (0.12 – Cadogan et al. 1997) various lepidopteran pests (0.23 – Gurr et al. 1999) beet armyworm (0.125 – Mulder and Prescott 1999a)
0.24	various beneficial arthropods (0.24 – Mulder and Prescott 1999a) beneficial arthropods (0.24 – Mulder and Prescott 1999b)	beet armyworm (0.24 – Mulder and Prescott 1999a) potato leafhopper (0.25 – Mulder and Prescott 1999b)

[1] Studies summarized in Appendix 6 with some efficacy studies omitted. The application rate in lb/acre and citation is given in parenthesis following the species or group. See text for discussion. A single asterisk (*) indicates transient or equivocal effects.

LIST OF APPENDICES

Appendix 1: Estimates of dermal absorption rates for tebufenozide

Table A1-1: Estimate of first-order absorption rate (k_a in hours^{-1}) and 95% confidence intervals.

Model parameters	ID	Value		
Coefficient for $k_{o/w}$	C_KOW	0.233255		
Coefficient for MW	C_MW	0.005657		
Model Constant	C	1.49615		
Number of data points	DP	29		
Degrees of Freedom (d.f.)	DF	26		
Critical value of $t_{0.025}$ with 26 d.f.[a]	CRIT	2.056		
Standard error of the estimate	SEE	16.1125		
Mean square error or model variance	MDLV	0.619712		
Standard deviation of model (s)	MSD	0.787218	$MDLV^{0.5}$	
X′X, cross products matrix		0.307537	-0.00103089	0.00822769
		-0.00103089	0.000004377	-0.0000944359
		0.0082	-0.0000944359	0.0085286

[a] Mendenhall and Scheaffer, 1973, Appendix 3, 4, p. A31.

Central (maximum likelihood) estimate:

$$\log_{10} k_a = 0.233255 \log_{10}(k_{o/w}) - 0.005657\ MW - 1.49615$$

95% Confidence intervals for $\log_{10} k_a$
$$\log_{10} k_a \pm t_{0.025} \times s \times (a'X'X\,a)^{0.5}$$

where a is a column vector of $\{1, MW, \log_{10}(k_{o/w})\}$.

NB: Although the equation for the central estimate is presented with $k_{o/w}$ appearing before MW to be consistent with the way a similar equation is presented by EPA, MW must appear first in column vector a because of the way the statistical analysis was conducted to derive X′X .

See following page for details of calculating $a'X'X\,a$ without using matrix arithmetic.

Appendix 1-1

The term $\mathbf{a'}\cdot(\mathbf{X'X})^{-1}\cdot\mathbf{a}$ requires matrix multiplication. While this is most easily accomplished using a program that does matrix arithmetic, the calculation can be done with a standard calculator.

Letting

$$\mathbf{a} = \{a\_1, a\_2, a\_3\}$$

and

$(\mathbf{X'X})^{-1} = \qquad \{$

$\{b\_1, b\_2, b\_3\},$
$\{c\_1, c\_2, c\_3\},$
$\{d\_1, d\_2, d\_3\}$
$\},$

$\mathbf{a'}\cdot(\mathbf{X'X})^{-1}\cdot\mathbf{a}$ is equal to

Term 1:	$\{a\_1 \times([a\_1 \times b\_1] + [a\_2 \times c\_1] + [a\_3 \times d\_1])\} +$
Term 2:	$\{a\_2 \times([a\_1 \times b\_2] + [a\_2 \times c\_2] + [a\_3 \times d\_2])\} +$
Term 3:	$\{a\_3 \times([a\_1 \times b\_3] + [a\_2 \times c\_3] + [a\_3 \times d\_3])\}.$

Table A1-2: Calculation of first-order dermal absorption rate (k_a) for tebufenozide.

Parameters	Value	Units	Reference
Molecular weight	352.48	g/mole	Table 2-1
$K_{o/w}$ at pH 7	17,800	unitless	Table 2-1
$\log_{10} K_{o/w}$	4.25		

Column vector *a* for calculating confidence intervals (see Worksheet A07a for definitions.)

a_1	1		
a_2	352.48		
a_3	4.25		

Calculation of $\mathbf{a'} \cdot (\mathbf{X'X})^{-1} \cdot \mathbf{a}$ - see Worksheet A07a for details of calculation.

Term 1	-0.0209811072		
Term 2	0.0389710295		
Term 3	0.0475467644		
$\mathbf{a'} \cdot (\mathbf{X'X})^{-1} \cdot \mathbf{a}$	0.0655	calculation verified in Mathematica 3.0.1.1	

$\log_{10} k_a = 0.233255 \log_{10}(k_{o/w}) - 0.005657\,\mathbf{MW} - 1.49615$ — Worksheet A07a

$\log_{10}$ of first order absorption rate (k_a)

Central estimate	-2.49869764236	±	$t_{0.025}$	×	*s*	×	$(\mathbf{a'} \cdot (\mathbf{X'X})^{-1} \cdot \mathbf{a})^{0.5}$
Lower limit	-2.91292499777	-	2.0560	×	0.787218	×	0.2559296778
Upper limit	-2.08447028695	+	2.0560	×	0.787218	×	0.2559296778

First order absorption rates (i.e., antilog or 10^x of above values).

Central estimate	0.003171775	hours^{-1}	
Lower limit	0.001222011	hours^{-1}	
Upper limit	0.008232462	hours^{-1}	

Appendix 1: Estimates of dermal absorption rates for tebufenozide *(continued)*

Table A1-3: Estimate of dermal permeability (K_p in cm/hr) and 95% confidence intervals.

Model parameters	ID	Value	
Coefficient for $k_{o/w}$	C_KOW	0.706648	
Coefficient for MW	C_MW	0.006151	
Model Constant	C	2.72576	
Number of data points	DP	90	
Degrees of Freedom (d.f.)	DF	87	
Critical value of $t_{0.025}$ with 87 d.f.[a]	CRIT	1.96	
Standard error of the estimate	SEE	45.9983	
Mean square error or model variance	MDLV	0.528716	
Standard deviation of model (s)	MSD	0.727129	$MDLV^{0.5}$
X′X, cross products matrix	0.0550931	-0.0000941546	-0.0103443
	-0.0000941546	0.0000005978	-0.0000222508
	-0.0103443	-0.0000222508	0.00740677

[a]Mendenhall and Scheaffer, 1973, Appendix 3, Table 4, p. A31.

NOTE: The data for this analysis are taken from U.S. EPA (1992), Dermal Exposure Assessment: Principles and Applications, EPA/600/8-91/011B, Table 5-4, pp. 5-15 through 5-19. The U.S. EPA report does not provide sufficient information for the calculation of confidence intervals. The synopsis of the above analysis was conducted in STATGRAPHICS Plus for Windows, Version 3.1 (Manugistics, 1995) as well as Mathematica, Version 3.0.1.1 (Wolfram Research, 1997). Although not explicitly stated in the U.S. EPA report, 3 of the 93 data points are censored from the analysis because they are statistical outliers: [Hydrocortisone-21-yl]-hemipimelate, n-nonanol, and n-propanol. The model parameters reported above are consistent with those reported by U.S. EPA but are carried out to a greater number of decimal places to reduce rounding errors when calculating the confidence intervals. See notes to Worksheet A07a for details of calculating maximum likelihood estimates and confidence intervals.

Appendix 1: Estimates of dermal absorption rates for tebufenozide *(continued)*

Table A1-4: Calculation of dermal permeability rate (K_p) in cm/hour for tebufenozide.			
Parameters	Value	Units	Reference
Molecular weight	352.48	g/mole	
$K_{o/w}$ at pH 7	17800	unitless	
$\log_{10} K_{o/w}$	4.25		
Column vector ***a*** for calculating confidence intervals (see Worksheet A07a for definitions.)			
a_1	1		
a_2	352.48		
a_3	4.25		
Calculation of $\mathbf{a' \cdot (X'X)^{-1} \cdot a}$ - see Worksheet A07b for details of calculation.			
Term 1	-0.0220577884		
Term 2	0.007751756		
Term 3	0.0564889197		
$\mathbf{a' \cdot (X'X)^{-1} \cdot a}$	0.0422	calculation verified in Mathematica 3.0.1.1	

$\log_{10} k_p = 0.706648 \log_{10}(k_{o/w}) - 0.006151\,MW - 2.72576$							Worksheet A07b	
$\log_{10}$ of dermal permeability								
Central estimate	-1.89061048	$\pm$	$t_{0.025}$	$\times$	*s*	$\times$	$\mathbf{a' \cdot (X'X)^{-1} \cdot a}^{0.5}$	
Lower limit	-2.18337858572	-	1.9600	$\times$	0.727129	$\times$	0.2054263858	
Upper limit	-1.59784237428	+	1.9600	$\times$	0.727129	$\times$	0.2054263858	

Dermal permeability			
Central estimate	0.0128644	cm/hour	
Lower limit	0.0065557	cm/hour	
Upper limit	0.025244	cm/hour	

Table A1-5: Summary of chemical specific dermal absorption values used for tebufenozide dermal absorption.

Description	Code	Value	Units	Reference/Source
First-order absorption rates (k_a)				
Central estimate	AbsC	0.0032	hour^{-1}	Table A1-2, values rounded to two significant figures
Lower limit	AbsL	0.0012	hour^{-1}	
Upper limit	AbsU	0.0082	hour^{-1}	
Zero-order absorption (K_p)				
Central estimate	KpC	0.013	cm/hour	Table A1-4, values rounded to two significant figures
Lower limit	KpL	0.0066	cm/hour	
Upper limit	KpU	0.025	cm/hour	

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference
ACUTE			
Mice (NOS)	>5.0 g/kg technical, single oral dose (NOS)	No treatment related mortalities or signs of toxicity at limit dose of 5.0 g/kg LD_{50}>5.0 g/kg	Hazleton and Quinn 1995b MRID 43781708
Rats, Crl:CD, 29 to 34-days old, weighing 73-101 g, 10 males and 10 females per dose group	0, 500, 1000, or 2000 mg/kg bw by gavage (single dose)	No treatment-related mortalities, clinical signs of toxicity, or effects on body weight at any dose level; no neurotoxic or neuropathological effects at any dose level. NOEL >2000 mg/kg bw (highest dose tested)	Swenson et al. 1994 MRID 43781706
Rats, CD, adults, 6 males and 6 females	single gavage dose of 5.0 g/kg bw Mimic® 240 LV	No mortalities, body weight effects, or clinical signs of toxicity. Acute oral LD_{50} >5.0 g/kg bw or 5000 mg/kg This study reveals the components of Mimic formulation. This information cannot be disclosed in this document.	Parno and Gingrich. 1994b MRID 44727702
Rats (NOS)	>5.0 g/kg technical, single oral dose (NOS)	"practically non-toxic;" no treatment-related mortalities or signs of toxicity at the limit dose of 5.0 g/kg LD_{50}>5.0 g/kg	Hazleton and Quinn 1995b MRID 43781708 (This appears to be a summary of Parno and Gingrich 1994b, detailed above)
SUBCHRONIC			
Dogs, 4 males and 4 females per dose group (NOS)	0, 150, 600, 2400, or 9600 ppm ai in diet for 2 weeks	No effects on body weight or food consumption and no clinical or gross observations of toxicity. No effects at 150 ppm ai (5.1 mg/kg bw/day) At ≥600 ppm ai, increased spleen weight was noted; at ≥2400 ppm ai, increased spleen-to-body weight ratio was noted; at 9600 ppm ai, additional adverse effects included decreased RBC, hemoglobin, and hematocrit values.	Hazleton and Quinn 1995b MRID 43781708 (Hazard evaluation/data summary)

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference
Dogs, one male and one female per dose group (NOS)	limit dose of 30,000 ppm ai (1000 mg/kg bw/day) in diet for 2 weeks	decrease in food consumption during week 1 but not week 2 (both sexes); decreased body weight (male), hematological effects (both sexes) included decreased RBC, hemoglobin, and hematocrit values, increased methemoglobin (females), reticulocytes, Heinz bodies, platelets and white blood cells. Treatment-related effects included increased bilirubin and other changes in serum chemistry (NOS) and increased spleen weights above the upper limit expected for this species. Limit dose of 30,000 ppm was considered too high to be used in 13-week study.	Hazleton and Quinn 1995b MRID 43781708 (Hazard evaluation/data summary)
Dogs, males, 4 per dose group (NOS)	0 or 1500 ppm ai technical for 6 weeks, followed by control diet (0 ppm) for additional 4 weeks; hematological parameters were measured in controls and treated dogs prior to treatment, at 6 weeks, at 8 weeks, and at 10 weeks	Study designed to examine reversibility of hematological effects after exposure to RH-5992 technical. After 6 weeks, hematological effects in treated dogs included decreases in RBC, hemoglobin, and hematocrit values; increases in methemoglobin, mean corpuscular volume, reticulocytes, and platelets. Complete recovery (i.e., effects on hemopoietic system returned to control values) by the end of the 2- or 4-week recovery period.	Hazleton and Quinn 1995b MRID 43781708 (Hazard evaluation/data summary)
Dogs, beagles, purebred, ~8-months old, 4 males and 4 females per dose group	oral administration by admixture of 0, 50, 500, or 5000 ppm (active ingredient) for 90 days; group mean compound consumption in mg/kg/day for 13 weeks was: 2.09, 20.13, or 202.42 mg/kg/day (FEMALES) and 2.05, 21.42, or 201.82 mg/kg/day (MALES)	Dietary concentrations of 500 or 5000 ppm had a direct effect on red blood cells, leading to low grade hemolytic anemia. NOEL = 50 ppm No clinical signs of toxicity were attributed to treatment; high dose males gained slightly less weight than controls but the difference was not statistically significant; high dose males and females ate slightly less food than controls but the difference was not statistically significant; treatment had no effect on food conversion efficiency; and no ocular lesions resulted from treatment.	Clay 1992 MRID 42436223

Appendix 2-2

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference

Additional Observations from Clay 1992 MRID 42436223:

Hematology: there were several statistically significant effects on hematological parameters (e.g., red blood cell count, mean cell volume, reticulocyte counts, methemoglobin, incidence of Heinz bodies, and platelet counts) in males and females exposed to 500 or 5000 ppm. The presence of Heinz bodies is considered to represent a direct effect on the RBC and led to increased destruction of RBC in liver and spleen.

Urinalysis: urine of treated males was darker than urine of controls in week 13; three high dose males had bilirubin present in their urine (consistent with destruction of red blood cells).

Organ weights: in high dose males, mean absolute spleen weight was 30% greater than that of controls (p≤ 0.05) and relative spleen weight was 44% greater (p≤ 0.01); in females there was a significant dose response in relative spleen weight (p≤ 0.05); no statistically significant differences in relative liver weight among treated dogs; in high dose females, there was a statistically significant dose response with respect to increased liver weight.

Various treatment-related effects indicative of low grade hemolytic anemia were observed in the liver (increased incidence of pigment in the Kupffer cells), spleen (increased hemopoiesis and increased sinusoidal engorement) and bone marrow (hyperplasia) of males and female exposed to 500 or 5000 ppm.

Animal	Dose/Exposure	Response	Reference
Mice, males, 8 per dose group (NOS)	0, 60, 200, 600, 2000 or 6000 ppm ai technical in diet for 2 weeks	No effects at ≤600 ppm; increased liver-to-body weight ratio at 2000 or 6000 ppm; increased liver weight at 6000 ppm (~1000 mg/kg bw/day); no adverse effects on survival, clinical chemistry, body weight or food consumption.	Hazleton and Quinn 1995b MRID 43781708 (Hazard evaluation/data summary)
Mice, Crl:CD-1, ~4-weeks old, 10 males and 20 females per dose group	0, 20, 200, 2000 or 20,000 ppm in the diet for 13 weeks	No mortality; no treatment related clinical, cageside, or ophthalmoscopic observations. **Body weight**: significantly decreased mean body weight values at weeks 0-13 in males at 200 or 2000 ppm and at weeks 0-4 and 0-13 in males at 20,000 ppm; no statistically significant differences in mean food consumption values among all dose groups.	Osheroff 1991a MRID 42436221

Appendix 2-3

Animal	Dose/Exposure	Response	Reference

Additional Notes on Osheroff 1991a MRID 42436221

Hematology: significant increases in reticulocyte and absolute reticulocyte counts (males and females at 2000 or 20,000 ppm), mean cell volume (males at 2000 or 20,000 ppm), mean cell hemoglobin (males and females at 2000 or 20,000 ppm), mean cell hemoglobin concentration (males at 2000 and males and females at 20,000 ppm), white blood cell count, corrected white blood cell count, and lymphocyte counts (females at 2000 ppm and males and females at 20,000 ppm), heinz bodies (males at 2000 ppm and males and females at 20,000 ppm), and segmented neutrophils (males at 2000 ppm and males and females at 20,000 ppm). Decreased erythrocyte counts in males and female at 2000 or 20,000 ppm (significant only in males), decreased myeloid/erythroid ratios in males and female at 2000 or 20,000 ppm (significant only in females), significant increases in methemoglobin values in males and females at 2000 or 20,000 ppm, significant increased mean alkaline phosphatase and potassium values in males at 2000 or 20,000 ppm and significantly increased mean total protein and calcium values in males at 20,000 ppm.

Organ weights: significant decrease in mean terminal body weight in males at 20,000 ppm, significantly increased mean absolute and relative liver and spleen weights in males and 2000 ppm and in males and females at 20,000 ppm.

Gross necropsy: increased incidence in enlarged spleen males and females at 2000 or 20,000 ppm, increased incidence or severity of pigment accumulation in liver, spleen and kidney as well as increased extramedullary hematopoiesis in spleen of males and females at 2000 or 20,000 ppm.

Animal	Dose/Exposure	Response	Reference
Rats, 6 males and 6 females per dose group (NOS)	0, 50, 250, 1000, 2500, or 10,000 ppm ai technical in diet for 2 weeks	No effects at 50 or 250 ppm target organ = hemopoietic system at 1000 ppm, observations included decreased RBC (females), hemoglobin (females), and hematocrit (both sexes); increased liver weight (females) and liver-to-body weight ratio (both sexes). at 2500 ppm, additional effects included increased spleen weight (females) and spleen-to-body weight ratio (females) at 10,000 ppm (~700 mg/kg/day), additional effects included decreased food consumption, body weight (males), RBC (males), and hemoglobin (males); increased spleen weight (males) and spleen-to-body weight ratio (males). Effects at higher doses generally more severe than those observed at lower doses; no effects on survival or body weight (females), and no clinical signs of toxicity or gross pathology	Hazleton and Quinn 1995b MRID 43781708 (Hazard evaluation)

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference
Rats, 10 males and 10 females (NOS)	0 or 20,000 ppm ai in diet for 4 weeks; (20,000 ppm approximates limit dose of 1000 mg/kg/day)	Decreases observed in body weight, body weight gain, food consumption, RBC, hemoglobin, and hematocrit. Males showed increased liver and spleen weights (absolute and relative to body weight). There were no effects on survival and no clinical or gross signs of toxicity. This study together with the 2-week range finding test was used to select doses for the 13-week study.	Hazleton and Quinn 1995b MRID 43781708 (Hazard Evaluation and toxicity summary)

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference
Rats, CD, ~4-weeks old, 10 males and 10 females per dose group	0, 20, 200, 2000, or 20,000 ppm in diet for 13 weeks	No mortality; no adverse neurobehavioral, clinical, ophthalmoscopic, or gross necropsy findings. **Body weight**: statistically significant decrease at weeks 4 and 13 in females at 2000 ppm and in males and females at 20,000 ppm; body weight gain values significantly decreased at weeks 0-4 and 0-13 in males and females at 2000 or 20,000 ppm; food consumption significantly decreased at weeks 1-4 in males and females at 2000 or 20,000 ppm. **Hematology**: significant decreases in mean erythrocyte count, hemoglobin , and mean cell hemoglobin values as well as significant increases in mean cell volumes in males and females at 2000 or 20,000 ppm; decreased hematocrit and platelet values and increased mean cell hemoglobin and reticulocyte values in 20,000 ppm females; decreased myeloid/erythroid ratio in 2000 ppm females (with slight but not significant decrease in males and females at 20,000 ppm); significant increases in mean glucose and globulin values in females at 20,000 ppm. **Organ weights**: significantly decreased terminal body weight value for females at 2000 ppm and for males and females at 20,000 ppm; increased absolute liver weight in females at 20,000 ppm; increased spleen-to-body weight values in males and females at 20,000 ppm; increased liver-to-body weight values in females at 2000 ppm and males and females at 20,000 ppm; increased liver-to-brain weight value in females at 2000 or 20,000 ppm. **Histomorphology**: increased severity of splenic pigmentation in males and females at 2000 or 20,000 ppm. **NOEL (dietary administration for 13 weeks) = 200 ppm**	Osheroff 1991b MRID 42436219 MRID 43781708 (data summary)

Appendix 2-6

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference

CHRONIC

Animal	Dose/Exposure	Response	Reference
Dogs, beagles, purebred, 6- to 7-months old, weighing: 7.00-10.55 kg (males) and 5.75-9.05 kg (females), 4 males and 4 females per dose group	oral administration by admixture of 0, 15, 50, 250, or 1500 ppm for 52 weeks. Based on measured food consumption, these dietary concentrations corresponded to doses of 0.4 to 0.7 mg/kg bw (15 ppm), 1.5 to 2.4 mg/kg bw (50 ppm), 6.4 to 11.3 mg/kg bw (250 ppm), and 42.8 to 71.1 mg/kg bw (1500 ppm)	No clinical signs of toxicity associated with treatment; no adverse effects at ≤50 ppm; slight reduction in body weight gain (in the absence of any effect on food consumption) in males at 1500 ppm. At 250 and 1500 ppm, a direct effect of treatment on red blood cells was indicated by the presence of Heinz bodies and an increase in levels of methemoglobin, which resulted in the increased destruction of red blood cells in the liver (histologically associated with an increase in Kupffer cell pigment) and spleen. The increased destruction of red blood cells most likely accounted for the statistically significant increase in liver/body weight ratio in males at 1500 ppm and the increased spleen weights in dogs exposed to 250 and 1500 ppm. Also consistent with the effect of increased red blood cell destruction is the increase in plasma bilirubin at 250 and 1500 ppm.	Richards 1992a,b MRID 42931203 MRID 42931204

Additional Notes on Richards 1992a,b:
Other adverse effects included decreases in red blood cell counts, hemoglobin concentrations, and packed cell volume, compensatory increased in red blood cell production, minimal hemopoiesis in the spleen and hyperplasia in the sternal and femoral bone marrow, and increases in platelet and reticulocyte counts. All of these effects, which were observed consistently at 1500 ppm and to a lesser extent at 250 ppm, are indicative of low grade hemolytic anemia.

The increase in methemoglobin levels evidenced a statistically significant dose-response relationship at weeks 13, 15, 21, 39, and 52. [Table 5.1, p. 86. Fiche of this table is very difficult to read. Durations are taken from section 3.7, p. 23.] Based on comparisons to the control group, however, only the high dose group male dogs had a statistically significant increase by the end of the study, 1.7% in exposed group compared to 0.9% in the control group.

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference
Mice, Crl:CD-1, ~6-weeks old, weighing 23-33 g (males) and 17-26 g (females), 60 males and 60 females per dose group	nominal dietary concentrations of 0, 5, 50, 500, or 1000 ppm ai for 18 months, corresponding to overall compound consumption of 1, 8, 78, or 155 mg/kg/day (males) or 1, 9, 94, or 186 mg/kg/day (females).	NOEL = 50 ppm [8 mg/kg/day (males) and 9 mg/kg/day (females). No oncogenic effects at dietary levels up to 1000 ppm (equivalent to intake of 155 and 186 mg/kg/day for males and females, respectively); no adverse effects on body weight, body weight gain, food consumption, or food efficiency; treatment related effects indicative of chronic toxicity included hematological changes and spleen histopathology at 500 or 1000 ppm. Decreased survival in males at 500 and 1000 ppm and in females at 1000 ppm was judged to be an equivocal finding based on historical control data and lack of associated pathologies.	Trutter 1992a MRID 42931205 Trutter 1992b MRID 42931206
Rats, CRL:CD, ~6-weeks old, 70 males and 70 females per dose group	0, 10, 100, 1000, or 2000 ppm in diet for 24 months (interim sacrifice at 12 months); overall compound consumption values for males: 0.5, 5, 48, or 97 mg/kg/day, and for females: 0.6, 6, 61, or 125 mg/kg/day	no treatment related effect on survival; no oncogenic effects; treatment-related effects indicative of chronic toxicity at 1000 or 2000 ppm included decreased mean body weight and body weight gains, hematological effects (e.g., decreases in mean erythrocyte count, hematocrit and hemoglobin counts), and spleen histopathology (e.g., statistically significant increase in spleen-to-body weight ratio in high dose females, likely related to hematology findings). NOEL = 100 ppm (5 and 6 mg/kg/day for males and females, respectively)	Trutter 1992c MRID 42931208

Appendix 2-8

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference

REPRODUCTION/TERATOLOGY

Animal	Dose/Exposure	Response	Reference
Rabbits, New Zealand white, pregnant females, 5.5- to 6-months old, 20 per dose group	0, 50, 250, 1000 mg/kg/day once daily by gavage on day 7-19 of gestation; vehicle: aqueous 0.5% (w/w) sodium carboxymethyl-cellulose	No treatment-related deaths or clinical signs of toxicity; no treatment-related effects on maternal body weight or food consumption; no signs of maternal or developmental toxicity at any dose level. NOEL = 1000 mg/kg/day (highest dose tested)	Swenson and Solomon 1992 MRID 42436227
Rats, Sprague-Dawley, pregnant females, 25 per dose group.	0, 50, 250, or 1000 mg/kg/day once daily by gavage on days 6-15 of gestation; vehicle: aqueous 0.5% (w/w) sodium carboxymethyl-cellulose	No mortality; no clinical toxicity or adverse findings at necropsy. **At 1000 mg/kg/day**: reduced maternal body weight gain on days 6-20 of gestation (after correction for gravid weight); decrease in relative food consumption on days 7-8 and 6-9 of gestation, significantly reduced ($p \leq 0.05$) on days 8-9 of gestation. No effects on litter averages for corpora lutea, implantations, sitter sizes, live fetuses, early and late resorptions, or the number of dams with any resorptions. No developmental effects occurred at the high (1000 mg/kg/day) dose. NOAEL = 250 mg/kg/day.	Hoberman 1991 MRID 42436225
Rats, Crj:CD, ~5-weeks old, 24 males and 24 females per dose group	0, 25, 200, or 2000 ppm in diet for two consecutive generations	no reproductive effects at concentrations ≤ 2000 ppm systemic toxicity observed in parental rats (i.e., adverse effects on hemopoietic system and body weight effects) at concentrations ≥ 200 ppm NOEL (for reproductive effects) = 2000 ppm ai (149-195 mg/kg/day in males and females, respectively) NOEL (for systemic toxicity) = 25 ppm ai (1.9-2.3 mg/kg/day for males and females, respectively)	Aso 1995 MRID 43797701 Hazleton and Quinn 1995b MRID 43781708 (Hazard evaluation and data summary)

Appendix 2-9

Appendix 2: Oral toxicity of tebufenozide to experimental mammals (subdivided as acute, subchronic, chronic and reproductive toxicity and sorted by species/duration).

Animal	Dose/Exposure	Response	Reference
Rats, CRL:CD, ~6-weeks old, 25 males and 25 females per dose group	0, 10, 150, or 2000 ppm in diet through two generations	NOEL (for reproductive effects) = 150 ppm (11.5-13.6 mg/kg/day for males and 12.8-14.5 mg/kg/day for females)	Danberry et al. 1993 MRID 42931207 Hazleton and Quinn 1995a MRID 43781707

Additional Details from Danberry et al. 1993: No treatment related mortality or clinical signs of toxicity in any generation at any dose level; ≤150 ppm did not cause effects on body weights or food consumption in any generation; 2000 ppm caused a decrease in body weight and food consumption in P_1 and P_2 males; histopathological changes in the spleen and toxicity of the hemopoietic system in rats of both sexes from both generations were consistent with the general pattern of toxicity observed in other non-developmental/non-reproductive studies

There were no treatment-related effects on mating or fertility in either generation at any dose level; there were no treatment related effects on reproduction in either generation at 10 or 150 ppm; **at 2000 ppm, there was an increased incidence of mortality of females during delivery (P_2), an increase in gestation length (P_2), a decrease in the mean number of implantation sites per female (P_2), and an increased incidence (equivocal) of pregnant females that did not deliver (P_1 and P_2).**

There were no treatment related effects on any offspring with respect to body weights, viability, malformations, or variations.

Hazleton and Quinn 1995a (MRID 43781707) conclude that dietary concentrations ≤2000 ppm tebufenozide do not cause reproductive effects in rats; NOEL = 149-195 mg/kg/day in males and females, respectively; NOEL for toxicity = 25 ppm (1.9-2.3 mg/kg/day in males and females, respectively

Appendix 3: Dermal, inhalation, and ocular effects of tebufenozide in experimental mammals

Animal	Dose/Exposure	Response	Reference
DERMAL			
Rats, CD, adults, 6 males and 6 females	2.0 g/kg bw undiluted Mimic®240 LV applied to shaved intact skin and occluded for 24 hours, after which the application sites were wiped with paper towels saturated with tap water and blotted dry.	No mortalities, clinical signs of toxicity, or body weight effects. Red stains observed on the fur surrounding the eyes and muzzle of several animals were attributed to test methods and use of collars. Skin irritation, manifested as erythema, edema, dessication, and scabs, was observed; however, necropsy revealed no gross changes. Acute dermal LD_{50} >2.0 g/kg bw Rohm and Haas classifies the test formulation as "PRACTICALLY NON-TOXIC by single dermal exposure" This study reveals the components of in the formulation. This information cannot be released	Parno and Gingrich 1994a MRID 44727703
Rats (NOS)	5.0 g/kg technical, single dermal application	"practically non-toxic;" no treatment-related mortalities or signs of toxicity at limit dose of 5.0 g/kg LD_{50}>5.0 g/kg	Hazleton and Quinn 1995b MRID 43781708 (Hazard evaluation/Toxicity summary)
Rats, CD, adults, 6 males and 6 females	5000 mg/kg bw undiluted Mimic®240 LV applied to shaved intact skin and occluded for 24 hours, after which the application sites were wiped with paper towels saturated with tap water and blotted dry.	No mortalities, clinical signs of toxicity, or body weight effects. Dessication at the application site affected several of the animals beginning on day 3 and continuing until day 9; necropsy revealed no gross changes. Acute dermal LD_{50} >5000 mg/kg bw Rohm and Haas classifies the test formulation as "PRACTICALLY NON-TOXIC by single dermal exposure" This study reveals the components of in the formulation. This information cannot be released	Parno 1997 MRID 44727704
Rats, 10 males and 10 females per dose group (NOS)	0 or 1000 mg ai/kg bw/day semi-occlusive 6-hour dermal exposure, 5 days/week for 4 weeks or 0, 62.5, 250, or 1000 mg ai/kg bw/day.	NOEL (dermal application for 4 weeks) = 1000 mg ai/kg bw/day No treatment-related effects on hematology or clinical chemistry parameters, organ weights, gross pathology or histopathology at any dose level	Hazleton and Quinn 1995b MRID 43781708 (Hazard Evaluation/data summary)

Appendix 3-1

Appendix 3: Dermal, inhalation, and ocular effects of tebufenozide in experimental mammals

Animal	Dose/Exposure	Response	Reference
Rats, Crl:CD, adults, 6 males and 6 females per dose group	Daily dermal applications of RH-75,992 2F formulation and RH75,992 technical or skin of rats for 4 weeks at doses up to and including 1000 mg ai/kg/day.	NOEL = 1000 mg ai/kg No treatment-related systemic effects; minor dermal irritation observed in females were attributed to RH-75,992 2F formulation solvent and not the active ingredient.	Morrison et al. 1993 MRID 42991507
Rabbits, New Zealand white, adults, 6 males	0.5 mL undiluted Mimic®240 LV applied to shaved intact skin and sites were semi-occluded for 4 hours, after which the application sites were wiped with paper towels saturated with tap water and blotted dry.	No mortalities or clinical signs of toxicity. At 1 hour, well-defined erythema was observed in all rabbits (6/6). Observed erythema ranged from well-defined to none among rabbits at 24, 48, and 72 hours but was no longer evident by day 7. Edema was not observed during the study. Rohm and Haas classifies the test formulation as slightly irritating to skin. This study reveals the components of in the formulation. This information cannot be released	Parno 1997 MRID 44727704
Guinea pigs, Hartley, young females, 20 treated, 10 positive controls, 10 naive controls	Skin sensitization protocol as detailed in the first row of the next page.	No significant erythema observed in any of the guinea pigs induced with mimic formulation; 100% incidence of erythema in positive control group; no erythema in naive control group. Mimic did not produce delayed contact hypersensitivity in guinea pigs in this study. This study reveals the components of in the formulation. This information cannot be released	Anderson and Shuey 1994

*Anderson and Shuey 1994 **Exposure details:***
Induction: treated guinea pigs received three 6-hour induction doses (1 dose/week for 3 consecutive weeks) of 0.4 mL undiluted Mimic®240 LV to shaved skin; positive controls received three 6-hour induction doses (1 dose/week for 3 consecutive weeks) of 0.4 mL DNCB (1600 ppm in 80% aqueous ethanol). **Challenge dose**: 2 weeks after the last induction dose, treated pigs received 0.4 mL undiluted Mimic®240 LV and positive controls received 0.4 mL DNCB (800 ppm in acetone). Naive control group received 0.4 mL undiluted Mimic®240 LV to shaved skin at one site and 0.4 mL DNCB (800 ppm in acetone) at a separate site.

Appendix 3-2

Appendix 3: Dermal, inhalation, and ocular effects of tebufenozide in experimental mammals

Animal	Dose/Exposure	Response	Reference
Guinea pigs, young adults, albino, 20 (test group), 10 (control and positive control groups), 5 (positive control-naive control).	Test material administered as 5% w/w mixtures for intradermal injection and as 25% w/w mixture in petrolatum for topical induction and challenge applications	No skin sensitization in guinea pigs treated with test material; sulfathizole (used for positive control group) was shown to be an extreme sensitizer.	Glaza 1993 MRID 42991506

INHALATION

Animal	Dose/Exposure	Response	Reference
Rats, 5 males and 5 females (NOS)	4.3 mg/L aerosol dust for 4 hours (NOS)	LC_{50}>4.3 mg/L (males) [0/5 deaths] LC_{50}>4.5 mg/L (females) [0/5 deaths] These were highest technically achievable concentrations.	Hazleton and Quinn 1995b MRID 43781708 (hazard evaluation)
Rats, Crl:CD, 6 males and 6 females	MIMIC wettable powder formulation. Mean aerosol concentration of 1.83 mg/L, nose-only exposure for 4 hours, followed by 14-day observation period	No mortality; no treatment-related clinical signs of toxicity or body weight effects; no treatment-related gross lesions observed at necropsy. LC_{50} >1.83 mg/L This study reveals the components of in the formulation. This information cannot be released	Bemacki and Ferguson 1994a MRID 44200306
Rats, CD, adults, 6 males and 6 females	4-hour nose only exposure to measured concentration of 1.33 mg/L Mimic®240 LV (nominal concentration = 178.2 mg/L The difference between the measured and nominal concentrations is attributed to the impaction of a portion of the aerosol on the interior surfaces of the exposure system.	No mortalities or body weight effects. Clinical signs included wet fur immediately after exposure, respiratory noise (1/6 males and 1/6 females), red-stained fur around eyes (1/6 males and 1/6 females), red-stained muzzle (1/6 males), tan-stained muzzle (5/6 males and 5/6 females). The tan stains (appearing to be test material) were attributed to poor positioning of the animals in the nose-only tubes. Tan stains, which appeared up to and including day 1 were not evident by day 2. Necropsy revealed the following changes: red pinpoint foci in the lungs (5/6 males, 1/6 females), slight to severe redness on all lobes of the lung (4/6 males and 6/6 females), which were considered to be consistent with irritation of the respiratory tract and judged to be treatment related. Combined male and female LC_{50} >1.33 mg/L	Bemacki and Ferguson 1994b MRID 44727705 This study reveals the components of in the formulation. This information cannot be released

OCULAR

Appendix 3-3

Appendix 3: Dermal, inhalation, and ocular effects of tebufenozide in experimental mammals

Animal	Dose/Exposure	Response	Reference
Rabbits (NOS)	direct application to corneal surface of eye or into conjunctival sac (NOS)	no irritation in eyes washed 30 or 60 seconds after dose or in treated eyes that remained unwashed RH-5992 technical calssified as "inconsequentially irritating to the eye."	Hazleton and Quinn 1995b MRID 43781708 (hazard evaluation and toxicity summary)
Rabbits, New Zealand white, adults, 6 males	0.1 mL undiluted Mimic®240 LV applied to conjunctival sac of one eye; untreated eye served as control. After 24 hour observation period, eyes irrigated with saline for approximately 60 seconds. Approximately 75% of test substance remained in contact with the eyes.	No mortality or clinical signs of toxicity. At 1, 24, 48, and 72 hours, positive corneal and conjunctival effects were observed in 2/6 rabbits; effects no longer evident by day 7. Rohm and Haas classifies Mimic®240 LV "MODERATELY IRRITATING" (i.e., a positive test that is reversible at $\geq$ 24 hours but $\leq$7 days.	Gingrich and Parno 1994s MRID 444727706

Appendix 3-4

Appendix 4: Toxicity of tebufenozide to birds after oral administration.

Animal	Dose	Response	Reference
ACUTE			
Bobwhite quail, 13-days old, 10 per dose group	0, 312, 625, 2500, or 5000 ppm a.i. in diet for 5 consecutive days followed by a 3-day recovery period. Food consumption was about 13% of body weight during the exposure period (Tables III and IV). Thus, the dietary concentrations correspond to doses of 0, 41, 81, 325, 650 mg/kg bw/day.	LD_{50} >5000 ppm a.i.	Fletcher. 1990a MRID 42436235
Ducks, Mallard, 8-days old, 10 per dose group	0, 312, 625, 1250, 2500 or 5000 ppm in diet for 5 consecutive days followed by a 3-day recovery period	LD_{50} >5000 ppm a.i.	Fletcher 1990b MRID 42436237
LONGER-TERM			
Bobwhite quail, 29-weeks old, five males and five females per dose group	0, 1470, or 2150 mg a.i./kg via gelatin capsules for 21 days.	No mortality, no signs of toxicity, and no statistically significant difference in body weights, compared with controls. No abnormal tissue alterations were observed at necropsy. Acute LD_{50} >2150 mg a.i./kg bw	Fletcher 1987 MRID 42436234
Ducks, Mallard, 25-weeks old, 16 males and 16 females per dose group	0, 100, 300, or 1000 ppm ai in the diet for 20 weeks	No mortalities or treatment related adverse effects at any dose level; no adverse effects observed on body weight, food consumption, or reproductive endpoints. NOEL = 1000 ppm ai	Beavers et al. 1993a MRID 42991503

Appendix 4-1

Appendix 4: Toxicity of tebufenozide to birds after oral administration.

Animal	Dose	Response	Reference
Bobwhite quail, 18-weeks old, 16 males and 16 females per dose group	0, 100, 300 or 1000 ppm ai in the diet for 20 weeks. Based on reported food consumption rates of about 15% of body weight (see special note below), the dietary concentrations correspond to doses of 0, 15, 45, and 150 mg/kg/day. See special note below.	No treatment-related mortalities, overt signs of toxicity, or effects on body weight or food consumption at any concentration. **Reproductive effects**: at 300 ppm, possible slight reduction in number of eggs laid (reflected in 14-day old survivors as % maximum eggs set and number of 14-day old survivors per hen per day A substantial drop in feed consumption was observed during weeks 8 and 9. At 1000 ppm, slight decreases in number of eggs laid and number of viable embryos. NOEL (for reproductive parameters) = 100 ppm	Beavers et al. 1993b MRID 42991501 Reinert et al. 1993a MRID 42991502

SPECIAL SUPPLEMENTAL NOTES ON BEAVERS ET AL. 1993b [MRID 42991501, MRID 42991502]
mg/kg bw doses: Average doses in units of mg/kg bw are not provided in the study. Table 2, p. 34. Average food consumption is estimated at 30 g per bird. There was a slight transient decrease food consumption at weeks 10 and 11 in all dosed animals and weeks 13/14 in the two higher dose groups. The magnitude of the decrease was about 16% to 33% below that of controls. The average body weights of the animals was about 200 g over the course of the study. Thus, food consumption is taken as 15% of body weight (30 g/200 g). The methods specifically state that food and water were available *ad libitum*. *"No attempt was made to quantify the amount of feed wasted by the birds, as the wasted feed is normally scattered and mixed with water and excreta."* (p. 16).

Effects: See Supplemental Table 1 at the end of this appendix.

Reinert et al. 1993a [MRID 42991502], which is a supplemental report indicates that two orders of magnitude difference between the NOEL for bobwhite quail (100 ppm) and mallard duck (1000 ppm) is not consistent and concludes that many of the endpoints in the bobwhite study are confounded by the usual variability in long-term studies and that the lack of dose-response in many parameters when judged against available data in avian studies does not support a conclusion of adverse effects at 300 ppm ai in the diet and that the NOEL probably approaches 1000 ppm, as supported in the mallard study.

Appendix 4: Toxicity of tebufenozide to birds after oral administration.

Animal	Dose	Response	Reference
Bobwhite quail, 18-weeks old, 15 males and 15 females per dose group	0, 150, 240, 385, or 615 ppm ai in diet for 20 weeks. Based on reported food consumption rates of about 8% of body weight (see special note below), the dietary concentrations correspond to doses of 0, 12, 19.2, 30.8, 49.2 mg/kg/day.	No treatment-related mortalities, overt signs of toxicity or effects on body weight or feed consumption; no apparent effects on reproductive endpoints. NOEL = 615 ppm (highest dose tested) LOAEC >615 ppm	Reinert 1995a MRID 43781701 Reinert 1995b MRID 43781702 (Supple-mental report) Reinert 1995c MRID 43781703 Supple-mental report of statistical analysis)

SPECIAL SUPPLEMENTAL NOTES ON REINERT 1995a,b [MRID 43781701 AND MRID 43781702]:
mg/kg bw doses: Average doses in units of mg/kg bw are not provided in the study. Table 3b, p. 24. Average food consumption is estimated at 16 g per bird. This is only about one-half of the food consumption in the Beavers et al. 1993b study - i.e., about 30 g/bird - summarized in the previous entry. The average body weights of the animals was about 200 g over the course of the study, similar to the body weights in the Beavers et al. 1993b study. Thus, food consumption is taken as 8% of body weight (16 g/200 g). The food consumption estimates did explicitly consider measurements of food wastage - i.e., food scattered from the container and not consumed. Ad libitum feeding is assumed but not specified.

Effects: See Supplemental Table 2 at the end of this appendix.

Appendix 4, Supplemental Table 1:
Details of reproductive parameters in bobwhite quail (from Beavers et al. 1993b, Table 3, p. 36)

Parameter	PPM in Diet			
	0	100	300	1000
Eggs Laid	714	769	570	508
Eggs Cracked	12	15	9	14
Eggs Set	627	680	496	435
Viable Embryos	595	616	451	367
Live 3-Week Embryos	592	609	451	367
Hatchlings	569	564	429	348
14-Day Old Survivors	544	516	387	322
Eggs Laid/Hen	48	48	38	36
Eggs Laid/Hen/Day	0.68	0.69	0.54	0.52
14-Day Old Survivors/Hen	36	32	26	23

Appendix 4, Supplemental Table 2:
Details of reproductive parameters in bobwhite quail (from Reinert 1995a, pp. 24-29)

Parameter	PPM in Diet				
	0	150	240	385	615
Eggs Laid	640	632	514	671	516
Eggs Cracked	2	2	1	0	0
Eggs Set	576	587	476	623	483
Viable Embryos - Day 5 Candeling	492	550	409	589	449
Viable Embryos - Day 11 Candeling	488	545	398	578	446
Live 18-Day Embryos	476	540	392	573	441
Hatchlings	449	474	345	522	408
14-Day Old Survivors	418	429	323	491	375
Eggs Laid/Hen	42.7	42.1	36.7	44.7	34.5

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Insects			
Honey bee, adult	0, 59, 117, and 234 µg/bee; 96 hour observation period.	Mortality rates in exposed bees were about 3.4% to about 5% and were less than control mortality (5.88%) NOEC = 234 µg/bee	Atkins 1990 MRID 42436244
Mite, predatory *Stethorus punctum*	Tests on larvae, pupae, and adults by 24-hour dry film exposures, with concentrations ranging from 9-90 ppm. Tests on eggs placed on treated leaves (92 ppm) Note: unclear if concentrations are concentrations of solutions leaves were dipped in or concentration on leaf material.	Not toxic to eggs, but survival of larva was reduced compared to untreated controls. Larval mortality likely due to contact with residues on leaf (not delayed effect of exposure during egg stage) In contact assay, tebufenozide was not toxic to adults and did not effect pupal survival. Less toxic than diflubenzuron.	Biddinger and Hull 1995
Tufted apple bud moth larvae (*Platynota idaeusalis*) [target species]	Dietary exposure.	7-Day LC_{50} = 1.63 ppm 14-Day LC_{50} = 1.12 ppm Somewhat lower LC_{50} values in sensitive laboratory strain.	Biddinger et al. 1998
Tufted apple bud moth larvae (*Platynota idaeusalis*) [target species]	Dietary exposure. 0.03 or 0.05 ppm	No effect on larval or pupal development. Decreased fecundity in matings when both sexes were exposed.	Biddinger and Hull 1999
Cydia pomonella codling moth [target species]	Dietary exposure.	LC_{50} = 0.025 ppm Dose-related decrease in number of viable eggs from exposed females, especially at concentrations > than the LD_{50}. No effect if males only were exposed. Dose-dependent decreased in time to emergence of adult insect from pupal case. Effect more pronounced in females than males.	Brown 1996
Hyssopus pallidus, Hymenopteran parasitoid on codling moth eggs	Exposure via codling moth exposed to up to 40 ppm tebufenozide in diet [24x LC_{50}]	No adverse effects on egg or larval development of parasitoid at 40 ppm tebufenozide [24x LC_{50}]	Brown 1996

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Ascogaster sp Hymenopteran endoparasitoid on codling moth eggs	Codling moth exposed to 40 ppm tebufenozide [24x LC_{50}]	LC_{50} = 0.07971 ppm, 3x LC_{50} values for moth	Brown 1996
Honey bee (*Apis mellifera*)	24-hour and 72-hour exposure by direct contact, indirect contact (test substance on filter paper) and inhalation to 0.1% v/v (equivalent to 1.05 kg/ha in 1000 L/ha) tebufenozide formulation Hoe 105540 SC (a 24% a.i. water soluble formulation) 3-hour (250 µg a.i./bee) feeding and 24-hour feeding (dose range approximately 2.4 to 800 µg a.i./bee) Note: for all contact and inhalation exposures, it is unclear is concentrations are given in terms of formulation or a.i. Authors state that 0.1% v/v is equivalent to twice the application rate	Direct exposure **24-hr**: 2% mortality in treatment group and 0% in controls **72-hr**: 14% mortality in treatment group and 12% in controls Indirect exposure **24-hr**: 0% mortality in treatment and control. **72-hr**: 10% mortality in treatment group, 8% in controls. Inhalation exposure **24-hr**: 0% mortality in treatment and 2% mortality in control **72-hr**: 10% mortality in treatment and control. Oral exposure **3-hr**: 0% mortality in treatment and control. **LD_{50} > 250 µg/bee** **24-hr**: 0% mortality in highest dose group. 2% mortality in controls. No dose-dependent mortality was observed. **LD_{50} > 800 µg/bee**. No behavioral effects noted for any route of exposure or duration of exposure.	Chan 1995 MRID 43797702
Honey bee (*Apis mellifera*)	tebufenozide formulation Hoe 105540 SC (a 24% a.i. water soluble formulation) applied at rate of 1.05 kg/300 L applied at rate of 0.2 kg/ha. [Appears to be given in terms of formulation, although this was not specifically stated]	Bee colonies tested in laboratory. No increased in treatment-related mortality was observed. No effects of treatment on flight activities or behavior. No effects on brood (as measured by dead pupae).	Chan 1995 MRID 43797702

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Trichogramma pretiosum (parasitic wasp)	Exposure to *T. pretiosum* by dipping parasitized host eggs of *Ephestia kuehniella* in solutions of tebufenozide. Eggs dipped for 5 seconds on tebufenozide solution of 25 g a.i./100 L.	Three different development stages of parasitized host eggs tested – egg-larvae, pre-pupae, and pupae. No significant increase in *T. pretiosum* mortality compared to untreated controls. Decreased development time was slightly significantly decreased for tebufenozide applied at the pupae stage (tebufenozide 9.68 days in control group and 9.35 day in tebufenozide group), but not when applied at the egg-larvae and pre-pupae stages. For parasite, parasitism capacity reduced when tebufenozide was applied at the egg-larvae and pre-pupae stages, but not when applied at the pupal stage,	Consoli et al. 1998

Mexican rice borer (*Eoreuma loftini*)	laboratory study. Exposure via leaves collected from sprayed field as follows:
	
	1996 season
	leaves collected 1 day after field application of low dose Confirm (0.14 kg a.i./ha) and high dose Confirm (0.2 kg a.i./ha). Insects were 1st instar larvae
	
	1997 season
	leaves collected 1 and 4 days after application of Confirm (rate of 0.28 kg a.i./ha). Insects were 2nd and 3rd instar larvae
		For the 1996 season
		Cumulative mortality as follows:
		   low dose: 34.4%
		   high dose: 39.4%
		   untreated control: 0%
		
		For the 1997 season
		For organisms exposed to leaves collected 1 day after field application: after 9 days of exposure, mortality was approximately 80% (data presented graphically). 100% mortality after 12 days of exposure
		
		For organisms exposed to leaves collected 4 days after field application: after 9 days of exposure, mortality was approximately 20% (data presented graphically). Mortality not assessed after 9 days.
			Legaspi et al. 1999

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
braconid parasitoid *Allorhogas pyralophagus*	exposure via leaves collected 1 and 4 days after field after applications of Confirm in 1996 and 1997. 1996: low dose 0.14 kg a.i./ha and high dose0.2 kg a.i./ha 1997: 0.28 kg a.i./ha	Using 1997 field treatments [according to figure 5 legend, p 809], no mortality was observed in for *A. pyralophagus* exposed to leaves (collected 1 day and 4 days after field application) for 4 and 24 hrs. Using 1997 field treatments [according to figure 6 legend, p 809], no difference was observed between control and high dose tebufenozide, but longevity was decreased for low dose tebufenozide.	Legaspi et al. 1999

Note on Legaspi et al. 1999: From the methods section, it appears that 2 application rates of Confirm were tested in 1996 and one was tested in 1997. However, results for 1997 are presented for low and high dose groups.

Species	Exposure	Effects	Reference
Beet army worm, 3rd instar (Lepidoptera: noctuidae)	tebufenozide (Confirm 2F) in food at 22.7 % a.i. (wt/wt) after exposure to diet for 120 hours	Susceptibility of field collected insects (9 strains) compared to ECOGEN laboratory strain using LC_{50} values ECOGEN LC_{50}: 17.6 ppm Field organisms LC_{50} values range from 39.7 to 176.3 ppm	Mascarenhas et al. 1998

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
predatory lacewing adults (*Chysoperla carnea*)	tebufenozide (TEB), 18, 90 and 180 ng/insect, applied topically [authors note that 90 mg/insect is the maximum field recommended (MFRD) dose]	Tebufenozide did not fecundity and egg fertility. In contrast, diflubenzuron reduced egg hatchability to 0% (compared to control 87%).

To explore differences, compared cuticle penetration, distribution and excretion of compounds. | Medina et al. 2002 |
| | Diflubenzuron (DBB) applied at 150 (2xMFRD) | Cuticle penetration:
  DFB 16%
  TEB 26%

Excretion:
  DFB 24.8% of penetrated amount excreted in feces in 7 days
  TEB aprpox, 50% of penetrated amount excreted in feces in 7 days

For DFB, only very small amounts of dose recovered in ovaries and deposited eggs. No TEB detected in ovaries or deposited eggs. | |
| predatory lacewing 3rd instar lavae (*Chysoperla carnea*) | Topical application of tebufenozide (TEB, Mimic 24% a.i.) applied at 0, 90 and 180 ng a.i./insect and diflubenzuron (DFB, 25% a.i.) applied at doses ranging from 0.5-75 ng a.i./insect

Authors note that for TEB, 90 ng/insect is the maximum field recommended dose (MFRD) | TEB had no effect on pupation, adult emergence, fecundity or egg fertility.

DFB LD_{50}: 2.26 ng a.i./insect. At the lowest dose tested (0.5 ng a.i./insect), no effect on fecundity or egg fertility compared to control

Presented results of cuticle penetration and excretion studies as summarized above for Medina et al. 2002 | Medina et al. 2003 |

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Indian meal moth (*Plodia interpunctella*)	dietary exposure of 1st instar lavae to tebufenozide (RH-5992) at concentrations of 0, 0.1, 1, 5, 10, and 25 ppm for up to 31 days	Larvae monitored for weight and mortality until metamorphosis. Weight gain: No effect on wt gain at concentrations up to 1.0 ppm. Exposure to 5 and 10 ppm results in decreased wt gain. Exposure to 25 ppm results in larval weight loss. Mortality: At concentrations of 0.1 and 1 ppm, no effect on mortality. Mortality increased compared to control at concentrations 5 and 10 ppm. 100% mortality at 25 ppm. In cell culture (PID2 imaginal disc line), exposure to 0.005 μM tebufenozide significantly increased glucosamine uptake (increase by 30% of control level).	Oberlander et al. 1998
spruce budworm (*Choristoneura fumiferana*)	not reported in Keller and Brown 1998a summary	RH-5992 is effective in inducing a incomplete molt when fed to worms prior to appearance of the endogenous ecdysteroid peak, but when administered after the peak. However, incomplete molts are observed for subsequent molts, presumably due to the persistence of tebufenozide in cells.	Palli et al. 1995, as summarized in Keller and Brown 1998a
predaceous insidium flower bug (*Orius inisidoisus*), parasitic wasp (*Cotesia plutella*)	Confirm applied cotton plants at an application rate of 0.125 lb a.i./acre. Insects were tested on plants 2 and 24 hours after application. Insects exposed to fresh foliar residues for 24 and 48 hours.	O. insidoisus: exposure to 2- and 24-hour leaves for 24 or 48 hours did not results in an increase in mortality compared to control insects. C. plutella: no significant increase in percent mortality compared to control exposed to 2-hour old leaves.	Pietrantionio and Benedict 1999

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
spruce budworm (*Chorisroneura fumiferana*)	1-100 ng/insect tebufenozide by ingestion	In 6th instar insects, treatment induced lethal precocious molt. Lack of development of new cuticle due to lack of gene expression of dopadecarboxylase. Effect observed in 100% of insects administered a dose of 70 ng. For 4th and 5th instars, 100% effect for lethal precocious molt was observed at lower dose (20 ng/insect) Topical exposure did not induce effects at doses up to 10,000 ng/insect.	Retnakaran et al. 1997a
spruce budworm (*Chorisroneura fumiferana*), 6th instar stage	Insects force-fed 0.1 μg a.i. tebufenozide (aqueous flowable RH-5992)	Effects observed at time points after exposure: 6 hr – insects stop feeding. 12 hr – head capsule slips partially. 24 hr – pronounced head capsule slippage and mid-dorsal split of old cuticle. Insect remains in this state and ultimately dies of starvation and dessication. Microscopy if integument showed hypertrophy of golgi complex and alterations in the cutlicular components, and organelles of epidermal cells.	Retnakaran et al. 1997b
two lacewing species – *Chrysoperla carnea* (Stephens) and *Micromus tasaniae* (Walker)	Petri dishes sprayed with tebufenozide (Minic 20 flowable liquid) at concentations of 0.08 to 0.8 % a.i.) and film left to dry. To test for acetylcholinesterase activity (AchE), insects were exposed for 2 and 24 hours. For Glutathione-S-transferase (GST), insects were exposed for 10 hours.	For both species, no inhibition of head AchE or whole body GST.	Rumph et al. 1997a

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
lacewing *Micromus tasaniae* (Walker) (3rd instars)	Test materials applied to petri dishes. Tebufenozide 7.44 $\mu g/cm^2$ (according to authors, this is 10x the recommended field rate). For tebufenozide-exposed larvae, effects in offspring were also examined, but offspring were not exposed to any test substance. Diflubenzuron (DFB) 0.07 $\mu g/cm^2$	Examined effects of tebufenozide and DFB on life-table parameters (sex ratio, longevity, sterility and fecundity) in adults derived from treated larvae. Tebufenozide: No mortality observed. No treatment effect for sex ratio, longevity or number of sterile pairs for either first or second generation. Total number of eggs in reduced by 30% in 2nd generation, but not 1st generation. Decreased in oviposition period for 1st generation (33.3 days) and 2nd generation (30.5 days), compared to control (39.8 days). Only 2nd generation change significant. No change in preoviposition period for either generation. DFB: Higher proportion of females in DFB (64.9%) compared to controls (53.0%). Longevity reduced for females in DFB (34.1 days) compared to controls (46.1 days). No treatment effect for in number of sterile pairs, although a strong trend observed toward an increase in infertility. Daily number of eggs reduced. Increased preoviposition period. Significant decrease in oviposition period.	Rumph et al. 1998
Codling moth (*Cydia pomonella*) – 3 strains	Tebufenozide (Confirm) dose range 10-10,000 ng/insect, applied topically	In susceptible strains of diapausing larvae, tebufenozide breaks the diapausing period and induces molting and reduces the pre-emergent period. In resistant strains, treatment did not break the diapausing state. LC_{50} values of various strains – Sv: 27.4 ng/insect Rv: 362 ng/insect Rt: 1570 ng/insect	Sauphanor et al. 1999

Appendix 5 - 8

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Larvae of *Galleria*, *Sarcophaga* and *Calliphora*	topical application of RH-5992 (dose range not specified in Keller and Brown 1998a summary)	*Galleria*: stimulation premature molt. $ED_{50} = 1.75$ µg/insect *Sarcophaga* and *Calliphora*: did not induce molt	Slama 1995, as summarized in Keller and Brown 1998a

Spodoptera exempta (Walker) (beet armyworm), *Spodoptera exigua* (Hubner) (beet armyworm), *Spodoptera littoralis* (Egyptian armyworm), *Mamestra brassicae* (cabbage moth), *Galleria mellonella* (greater Wax moth)	Exposure by topical or oral routes. Topical application of 0.01 to 40,000 ng/insect. Oral exposure by feeding leaves or prey dipped in tebufenozide solutions or tebufenozide in honey water
	
	(technical grade tebufenozide)
		S. exempta
		LD_{50} (topical application):
		  6.75 mg/insect for 6th instar
		LC_{50} (fed dipped leaves - values are concentration of test material leaves were dipped in)
		  3rd instar 0.034 mg/L
		  4th instar 0.095 mg/L
		  5th instar 0.085 mg/L
		  6th instar 0.084 mg/L
		
		S. exigua
		LD_{50} (topical application):
		  59.2 mg/insect for 5th instar
		LC_{50} (fed dipped leaves)
		  1st instar 9.7 mg/L
		  2nd instar 10.5mg/L
		  3rd instar 8.5mg/L
		  4th instar 10.0 mg/L
		  5th instar 2.5 mg/L
		Dose-dependent decrease in fecundity following oral exposure to tebufenozide in honey water (1, 10, and 100 mg/L), although all deposited eggs were viable
		
		S. Littoralis
		LD_{50} (topical application):
		  11.02 mg/insect for 6th instar
		
		M. brassicae
		LD_{50} (topical application):
		  8.53 mg/insect for 6th instar
		
		G. mellonella
		LD_{50} (topical application):
		  571 mg/insect for 6th instar
		
		For Lepidoptera larvae, tebufenozide induced lethal molt within 24 hours of exposure. Other effects included inhibition of weigh gain and feeding, extrusion of hindgut, and loss of hemolymph.
			Smaggje and Degheele 1994a

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
larvae of *Leptinotarsa decemlineata* (Colorado potato beetle), *Diabrotica virgifera virgifera* (western corn rootworm), *Locusta migratoria migratoria* (migratory locust), and nymphs of *Podisus sagitta* (predatory stink bug)	Exposure by topical or oral routes. Topical application of 0.01 to 40,000 ng/insect. Oral exposure by feeding leaves or prey dipped in tebufenozide solutions or tebufenozide in honey water (technical grade tebufenozide)	No activity observed in any species at any dose or concentration tested.	Smagghe and Degheele 1994b
Spodoptera exempta (Afrian army worm), *Spodoptera exigua* (beet armyworm), *Lepinotarda decemlineata* (Colorado potato beetle)	For LC_{50} determination, insects were fed leaves dipped in tebufenozide (technical grade) solutions.	**LC_{50} values** (last instars) *S. exempta*: 0.034 mg/L *S. exigua*: 2.5 mg/L *L. decemlineata*: no mortality at concentrations up to 50 mg/L. At 100 mg/L, sings of neurotoxicity (tremor and paralysis) were noted. For *S. exempta* and *S. exigua*, dose-dependent decreased in larval weights. No affect of treatment on larval weight for *L. decemlineata*. Resistance of *L. decemlineata* and differences in sensitivities of *S. exempta* and *S. exigua* apparently not due to differences in pharmacokinetics. All three species showed similar pharmacokinetic parameters for absorption, excretion, distribution and metabolism of tebufenozide	Smagghe and Degheele 1994b

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Podisus nigrispinus and *P. Maculiventris* (predatory soldier bugs)	nymphs exposed orally to RH-5992 via feeding on larvae of *Spodoptera exigua* treated with 20 μg/larvae or in drinking water (100 mg/L) or exposed topically to up to 100 μg/nymph. Adults treated orally via feeding on larvae of *Spodoptera exigua* treated with 20 μg/larvae or in drinking water (100 mg/L)	No effect in either species for any exposure. No chemosterilizing effects observed in adults	Smagghe and Degheele 1995, as summarized in Keller and Brown 1998a
Cotton leafworm (*Spodoptera littoralis*), laboratory strain and field strain	tebufenozide (RH-5992 2F flowable) For repeated exposures to induce tolerance, exposure was dietary via leaves dipped in 0.6 mg a.i./L tebufenozide solution. For LC_{50} determination, tebufenozide applied uniformly to food [unclear if concentrations are final concentration in food or concentration of fluid applied to food.]	Repeated exposure over 5 generations did not result in the development of tolerance to tebufenozide. For 3rd instar insects, laboratory strain (LC_{50} 2.47 mg/L) was more susceptible than the field strain (LC_{50} 11.31 mg/L).	Smagghe and Degheele 1997
Spodoptera exigua (beet armyworm) and *Leptinotarsa decemlineata* (Colorado potato beetle)	Dietary exposure via leaves dipped in solution of 3 mg a.i./L tebufenozide (technical grade) for *S. exigua* and 50 mg a..i/L tebufenozide	*S. exigua*: In control insects, major hemolymph ecdysteroid peaks appeared ~3-4 days. After treatment with tebufenozide, hemolymph ecdysteroid peaks was abolished. Treatment resulted in decreased weight gain. Typical precocious molting observed. *L. decemlineata*: In control insects, major hemolymph ecdysteroid peaks appeared ~8-9 days. Peak unaffected by tebufenozide treatment. No affect of treatment on larval weight gain. No precious molting observed.	Smagghe et al. 1995

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Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Chrysodeixis chalcites (tomato looper), last instar	exposure to diet containing 100 µg a.i./g diet tebufenozide RH-5992 2F	Symptoms of premature molting observed within 12 hours of treatment. Significant reduction in larval weight and feeding. Ultrastructural changes of the integument included increase in endoplasmic reticulum, hypertrophy of golgi complex, increase in nuclear volume, numerous oval and elongated mitochondria. Prothoracic gland cells were reduced in size, show loss of cell organelles, and autophagic vacuoles appeared. In foregut epithelium, prominent vacuoles formed and most cell oragnelles disappeared. Ultrastructural changes also observed in muscle cells, with absent mitochondria.	Smagghe et al. 1997
Spodoptera exigua (beet armyworm)	Exposure via artificial diet with concentrations of tebufenoxide varying according to generation. G_{0-5}: 0.5 mg/L G_{6-10}: 1 mg a.i./L $G1_{1-12}$: 2 mg a.i./L For disposition studies, all insects were exposed to the same amount of test material (20,000 dpm) consumed on leaf material.	Continuous exposure of all larval instars to LC_{25} doses for over 12 generations revealed no loss in susceptibility for up to 5 generations. From G_4 onwards, generation-dependent reduction in oviposition. For G_4, 65% of G_0 oviposition, for G12, 0% oviposition. Higher tissue concentrations of 14C-tebufenozide in hemolymph, carcass, and gut in susceptible larvae compared to G_0 larvae. All insects were exposed to the same amount of test material (20,000 dpm consumed on a leaf).	Smagghe et al. 1998
Spodoptera exigua (beet armyworm) and *Ostrinia nubilalis* (European corn borer)	*Spodoptera exigua* exposed to tebufenozide in diet. 50 µL of solution containing 1 mg/L tebufenozide (50 ng) added to artificial diet in culture dish for exposure to 1 insect. *Ostrinia nubilalis* exposed to tebufenozide (0, 10, 25, 50, 200, 300, and 400 ng/insect) by injection.	*Spodoptera exigua* (last instar): Chitin formation in cuticle was increased in tebufenozide treated insects compared to controls. Treated insects died by day 3 after exposure *Ostrinia nubilalis* (day-1 male pupae): Tebudenzide exposure prevented the completion of adult development and eclosion. Time to death decreased with increasing dose. Tebufenozide exposure induced premature chitin synthesis in male claspers.	Smagghe et al. 1999a

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Spodoptera exigua (beet armyworm), last instars	Tebufenozide applied topically to individual insects. Mortality counts made 7 days after exposure.	LD_{50} = 7.06 mmole/insect	Smagghe et al. 1999b
Cydia pomonella (codling moth)	Exposure of adults to surfaces treated with tebufenozide solution (360 ppm*) throughout their lives, including mating and ovipositing).	Continuous exposure to tebufenozide-treated surfaces resulted in significant reduction in number of eggs laid (control, 74.5 eggs; treatment 39.6 eggs) and number of eggs hatched (control, 58.4%; treatment, 6.6%).	Sun and Barrett 1999
	Recently emerged moths exposed to treated surfaces (360 ppm*) for 24 hours, then mated with unexposed partner (oviposit on non-treated surface)	24-hour exposure of females mated to unexposed males resulted in reduction in fecundity (control, 97.7 eggs; treatment 26.8 eggs) and fertility (control, 86.3%; treatment, 78.7%). No effect if exposed male was mated with unexposed female	
	tebufenozide was RH-5992, 2F (flowable)		
	* authors state that this is the recommended field rate		
Orius laevegatus (predatory bug)	exposure to plates sprayed with tebufenozide at the manufacturers recommended rate	No effect on development of nymphs or on oviposition.	van de Veire et al. 1996, as summarized in Keller and Brown 1998a
Gypsy moth [target species]	Tebufenozide applied to branches of oak trees at rate of "237 mL per 189 L final solution (label recommends 8 oz per 50 gal solution per acre), with 0.25 5 (v/v) Bond sticker".	Laboratory-reared gypsy moth larvae (1st, 2nd, 3rs, and 4th instars studied separately) were placed in bags and tied onto tips of treated branches 1 hour after spraying. Larvae were exposed for 7-21 days. Same protocol was followed for larvae applied to branches 1, 2, 7, 14, 21, 28 and 35 days after spraying.	Webb et al. 1998
	Difubenzuron (DFB) "Dimilin 25W at 237 mL per 378 L final solution, without added sticker".	For the exposure 1-hour post-application, 100% mortality observed for all insects after 21 days of exposure. Similarly, 100% mortality observed for all "aged" residues.	
		DFB also showed very high efficacy, except for 69% mortality on 14-day residue. However, all other DFB aged residues resulted in 100% mortality.	

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
Epiphyas postvittana (lightbrown apple moth)	larvae exposed to tebufenozide (Mimic 70W) in food at concentrations of 0, 0.5, 1. 1.5, 2, 2.5, 3, 10, 30, 100, and 200 ppm.	Dose-mortality response determined at each larval stage. 1st instar: no survival to pupation at concentrations $\geq$1.5 ppm 3rd instar: no survival to pupation at concentrations $\geq$2.5 ppm 5th instar: dose-related decrease in survival to pupation. In 200 ppm exposure group, 14.8% survival. Time to mortality was less than in1[st] and 3rd instars. Mortality increased with increasing exposure time. Time to mortality for 3rd and 5th instars decreased when insects were exposed at 40∘C compared to 20∘C. 3rd instars more susceptible at higher temperature than 5th instars.	Whiting et al. 1999

Soil Invertebrates

Species	Exposure	Effects	Reference
Earthworm *(Dendrobaena octaedra)*, 40 per dose	Deciduous leaves at 0 (untreated), 10X and 100 X EEC for 12 weeks. 55.4 ppm and 554 ppm based on reported EEC of 5.5461 mg/kg (equivalent to the application rate of 70 g/ha).	No effects on growth or reproduction (numbers or proportion hatching)	Addison 1996
Collembola (*Folsomia cundida*, *F. nivalis*, *Onychiurus parvicornis*, and *Hypogastrura pannosa*)	1996Coniferous substrate at 72.1 µg/g (ppm) organic matter for 8 to 10 weeks	No effect on survival or reproduction.	Addison 1996
Round worm larvae *(Ascaris suum)*	RH-5992 at concentrations in media of 5 and 50 ng/mL	Treatment had a biphasic effect on larval growth after 24-hour, premolt exposure – low concentrations (5 ng/mL) increase growth. Higher concentrations decreased growth ($\geq$ 50 ng/mL)	Fleming 1998, as summarized in Keller and Brown 1998a

Appendix 5. Toxicity of tebufenozide to terrestrial invertebrates (standard toxicity studies and microcosm studies).

Species	Exposure	Effects	Reference
earthworm (*Eisenia foetida*)	14-day exposure to RH-5992 at soil concentrations of 0, 61, 140, 270, 580, and 1000 mg a.i/kg (Although not specified, assume this is kg soil).No effect on survival at any concentration tested.	14-day LC50 > 1000 mg ai/kg 14-day NOAEC >1000 mg ai/kg	Garvey 1992, as cited in Keller 1994 (MRID 43367001)

Appendix 6. Terrestrial field/mesocosm studies on tebufenozide

Application	Species Examined	Effects	Reference
Mimic 2F, 0.03 lb a.i./acre in mixed oak forest, May 1994	Gypsy moth; Other macrolepidoptera richness and abundance	Examined effect of treatment on richness and abundance of arthropod family and macrolepidoptera. Sampling conducted May-Aug 1994 and May-Aug 1995. Marginal decrease in gypsy moth populations (not statistically significant compared to control plots). Nontarget arthropod richness and abundance: except for macrolepidoptera families, no effect of treatment for either sampling year. Significant decrease in the microlepidopteran Gelechiidae (p=0.02) in treatment year but not following year Marginal (p=0.07) decrease in sap-feeding Tingidae in treatment year but not following year. Macrolepidoptera richness: no effect of treatment in either sampling year (compared to control). Macrolepidoptera abundance: decreased during the last 8-13 weeks of 1994, but not different from control in the first 1-7 weeks of 1994 or for any sampling period in 1995.	Butler et al. 1997

Appendix 6. Terrestrial field/mesocosm studies on tebufenozide

Application	Species Examined	Effects	Reference
Mimic 2F, 0.06 lb a.i./acre in mixed oak forest, May 1994		Examined effect of treatment on richness and abundance of arthropod family and macrolepidoptera. Sampling conducted May-Aug 1994 and May-Aug 1995. Marginal decrease in gypsy moth populations (not statistically significant compared to control plots). Nontarget arthropod richness and abundance: except for macrolepidoptera families, no effect of treatment for either sampling year. Significant decrease in the microlepidopteran Gelechiidae (p=0.02) in treatment year but not following year Marginal (p=0.07) decrease in sap-feeding Tingidae in treatment year but not following year. Macrolepidoptera richness: decreased during the first 1-7 weeks after treatment in 1994 and during the first 1-8 weeks of the 1995 sampling period (compared to control). Macrolepidoptera abundance: decreased for the 1994 season and for the first 1-8 weeks of 1995 season.	Butler et al. 1997

Additional Notes on Butler et al. 1997: Some macrolepidoptera (e.g., *Melanolophia canadaria*) were relatively insensitive while others (*Lophocampa caryae* [Hickory Tussock moth]) were highly sensitive.

Application	Species Examined	Effects	Reference
Mimic 2F, 70 and 140 g/ha [0.06 and 0.12 lb a.i./acre]	Spruce budworm	Larval survival not significantly decreased at one application of 70 g/ha. Significant reductions at two applications at 70 g/ha or one application at 140 g/ha. Phenological development and larval and pupil weights significantly decreased in treated budworms compared to untreated controls.	Cadogan et al. 1997

Appendix 6. Terrestrial field/mesocosm studies on tebufenozide

Application	Species Examined	Effects	Reference
Mimic, tested on apple plots in Australia 1994/1995 season: 8 applications of 15 g a.i./100 L applied by air-blast sprayer at 1720 L/ha [258 g a.i./ha or 0.23 lb/acre] 1995/1996 season: 9 applications of 10.5 g a.i./L applied by air-blast sprayer at 1720 L/ha [180.6 g a.i./ha or 0.16 lb/acre]	lepidopteran pests and nontarget arthropods and	Note: no untreated control plot. All comparisons were made to plots treated with other insecticides (azinphos-methyl and fenoxycarb). All plots treated with Mimic showed effective control over lepidopteran pests (codling moth, lightbrown apple moth, and early seasons caterpillars) Populations of natural enemies (increased spiders, lacewings, and the specialist preditor mite *Stethorus* spp. adults and larvae.	Gurr et al. 1999
Mimic 240 LV. 0.07 a.i. kg/ha. Two aerial applications spaced 4 days apart in June 1994. Ontario Canada	Tennessee warbler nests, 6 in control plot and 5 in Mimic treated plot. Monitored number of eggs laid, percent hatch and growth of the hatchlings	Decreases in both the average number of eggs per nest (6.3 in the control area and 5.8 in the treated area) as well as the percent hatch (97.4% in the control area and 89.7% in the treated area). Based on the number of eggs, the differences in hatching were 37/38 in control plot and 26/29 in treated plot. Using the Fisher Exact test, the p-value is 0.21 – i.e., not statistically significant. Decrease in brooding time and increase in foraging times in Mimic treated plot were probably associated with decrease in prey.	Holmes 1998
Confirm 70W RH-5992 wettable powder applied to sugar cane plots in Texas. For the 1996 season, two application rates: 0.14 kg a.i./ha and 0.2 kg a.i./ha [0.12 lb/acre and 0.18 lb/acre]. For the 1997 season, 0.28 kg a.i./ha [0.25 lb/acre]	Mexican rice borer (*Eoreuma loftini*)	For all application rates for the 1996 and 1997 growing seasons - Treatment did not decrease the damage to cane caused by *E. Loftini* in either growing season. No increase in cane juice yield or quality in either growing season.	Legaspi et al. 1999

Appendix 6. Terrestrial field/mesocosm studies on tebufenozide

Application	Species Examined	Effects	Reference
Confirm 2F applied to plots of peanuts at rates of 0.125 and 0.24 lb a.i./acre. Treatment applied on Aug 7, 1998. Plots monitored on days 2, 5, 7, 10, 14 and 20 after application.	defoliating caterpillars and beneficial arthropods (not specified)	For defoliating caterpillars, the only decreased in numbers was observed for the high dose Confirm on day 3 (9% of control) after treatment. Only decrease in beneficial arthropods observed for low dose Confirm (315 of control) on Day 3 after treatment but not on subsequent days (5 to 15 DAT). For beet army worm, numbers were decreased for low (6% of control) and high (5% of control) application rates on day 3 after treatment.	Mulder and Prescott 1999a
Confirm 2F applied to plots of peanuts at 0.25 lb a.i./acre. Treatment applied on Aug 7, 1998.	potato leafhopper, defoliating caterpillars (corn earworm, beet armyworm, rednecked peanutworm, and beneficial arthropods (not specified)	Potato leafhopper numbers increased on day 14 after treatment (220% of control), but not days 7 and 20 Number of total defoliating caterpillars decreased on day 3 (52% of control) and day 7 (14% of control) after treatment. Number of beet aryworms decreased on day 7 (0% of control) after treatment. Number of beneficial arthropods not decreased at any time point.	Mulder and Prescott 1999b
Greenhouse study. Tebufenozide (RH-5992-2F) applied at 35, 70, 140 and 280 g a.i./ha to potted white spruce trees. [0.03, 0.06, 0.12, and 0.24 lb/acre]	spruce budworm (*Chorisroneura fumiferana*) exposed to trees for 10 days	Evaluated effectiveness of treatment by mortality and feeding rate of 4th instar insects (by counting number of droppings, i.e., frass pellets). After 10 days exposure, mortality was not increased compared to controls for any treatment group. However, feeding inhibition was apparent and similar for all treatment groups.	Retnakaran et al. 1997a

Appendix 6. Terrestrial field/mesocosm studies on tebufenozide

Application	Species Examined	Effects	Reference
Tebufenozide applied (RH-5992-2F) applied at 35, 70, 140 and 280 g a.i./ha [0.03, 0.06, 0.12, and 0.24 lb/acre] to 0.1 ha plots of white spruce trees in Zee Casault, Gaspe, Quebec.	spruce budworm (*Chorisroneura fumiferana*)	For plots treated with $\geq$70 g a.i./ha, population reduction was 100% For plots treated with 35 g a.i./ha, population reduction was 95%. For all tebufenozide treated plots, defoliation was 1-2%, compared to 13-16% in control plots.	Retnakaran et al. 1997a
Tebufenozide applied to apple plots in New South Wales, Australia.. Treatments applied between Nov to Feb over the 1992-1993 and 1993-1994 growing seasons. In each season, 8 applications of Mimic at rate of 15 g a.i./100 L (volume/acre or ha not indicated) using conventional air-blast sprayer. No untreated control plots.	Several species - codling moth, early fruit caterpillars (not specified), lightbrown apple moth, the predatory mites *Typhlodromus pyri* and *Typhlodromus occidentalis*, spiders (*Stetorus* spp) and apple dimpling bug nymphs (*Campylomma liebknechti*)	Comparisons of the effects of tebufenozide were made to 2 other treatments: azinphos-methyl and fenozycarb. No differences between treatments for fruit damage due to codling moth or early fruit caterpillars in either season. In the 1992-1993 seasons only, tebufenozide more effective than fenoxycarb on controlling damage due to lightbrown apple moth. Tebufenozide was ineffective in suppressing populations of the phyoseiids *Typhlodromus pyri* and *Typhlodromus occidentalis*. Compared to azinphos-methyl treatment, numbers of spiders (*Stetorus* spp) and apple dimpling bug nymphs (*Campylomma liebknechti*), numbers were higher in the tebufenzide-treated plots.	Valentine et al. 1996

Appendix 6. Terrestrial field/mesocosm studies on tebufenozide

Application	Species Examined	Effects	Reference
balsam firm tree plots in Newfoundland One application tebufenozide (Mimic) applied at a rate of 65.1 g a.i. in 1.86 L/ha [authors also refer to this dose as 70g a.i./ha equivalent to 0.06 lb/acre] Two applications tebufenozide (Mimic) at rate of 33.4-35.4 g a.i in 1.91-2.02 L/ha to [authors also refer to this dose as 35 g a.i./ha equivalent to 0.03 lb/acre]	eastern hemlock looper	One higher dose application: • 9/10 plots showed reduction of loopers. • 9-11 days post-treatment, 3-93% reduction. • 3 weeks post-treatment 8-100% reduction. • Pupal populations reduced 8-99% • Defoliation of year-old foliage 10-51% (control plots 35-65%) and current-year foliage 0-16% (control plots 15-39%). Two lower dose applications: • 9-11 days post-treatment, in general, >50 % reduction. • 3 weeks post-treatment, in general >60% reduction. • Pupal populations reduced 76-100% • Defoliation of year-old foliage reduced 1-33% (control plots 35-65%) and current-year foliage reduced 0-8% (control plots 15-39%). For both treatments, plots with poor efficacy were associated with low foliar deposition, with deposits <1.5 μg/g foliage (deposition measured for each plot) associated with ineffective control.	

Appendix 7: Toxicity of tebufenozide to fish.

Species	Exposure	Response	Reference
ACUTE			
Bluegill sunfish (*Lepomis macrochirus*), mean wt = 0.32 g, mean length = 24 mm, juveniles, 10 fish/dose group	nominal concentrations of 0, 0.5, 1.0, 2.0, 5.0, 10.0, 25.0, or 100 mg ai/L; mean measured concentrations of 0, 0.39, 0.90, 2.2, 4.0, 5.7, 9.4, or 18 mg ai/L (ranging from 18-100% of nominal concentrations) for 96 hours under static conditions	No toxicity observed at concentrations $\leq$0.39 mg ai/L 96 hr LC_{50} = 3.0 mg ai/L (95% CI = 2.2 and 4.0 mg ai/L) NOEC = 0.39 mg ai/L	Graves and Smith 1992b MRID 42436239
Rainbow trout (*Oncorhynchus mykiss*), juveniles, mean wet wgt = 0.39 g, mean standard length = 28mm, 2 replicates of 10 per dose group	nominal concentrations of 0, 0.5, 1, 2, 5, 10, 25 or 100 mg ai/L; mean measured concentrations of 0, 0.42, 0.84, 1.9, 4.7, 7.2, 10, or 17 mg ai/L for 96 hours under static conditions	96 hr LC_{50} = 5.7 mg ai/L (95% CI = 4.7 and 6.5 mg ai/L) NOEC = 1.9 mg ai/L no signs of toxicity at concentrations $\leq$1.9 mg ai/L; mortality data from the highest dose group was not used to calculate the LC_{50} values.	Graves and Smith 1992c MRID 42436240

Appendix 7-1

Appendix 7: Toxicity of tebufenozide to fish.

Species	Exposure	Response	Reference
LONGER-TERM			
Fathead minnow (*Pimephales promelas*), newly fertilized eggs (<24 hours after fertilization) used to initiate full life cycle study, 4 replicates of 25 animals per dose group.	mean measured concentrations of 0, 0.048, 0.090, 0.18, 0.35, or 0.72 mg ai/L (ranging from 92-100%) of nominal concentrations (0.048, 0.095, 0.19, 0.38, or 0.75 mg ai/L) under flow-through conditions. Both untreated and vehicle (acetone) control groups were assayed.	No effects on egg hatchability, parental generation growth, reproductive activity, or F_1 generation survival at any test concentration. Parental generation **survival was significantly decreased** at the two highest dose levels (0.35 and 0.72 mg ai/L): mean survival = **66% at 0.35 mg ai/L** (mortality = 22/25, 20/25, 7/25, and 17/25 in replicate groups A,B,C, and D, respectively) and **33% at 0.72 mg ai/L** (mortality = 9/25, 17/25, 3/25, and 4/25 in replicate groups A,B,C, and D, respectively).	Rhodes and Leak 1996 MRID 44221901 Reinert et al. 1999 MRID 44831501
Fathead minnows (*Pimephales promelas*), 30 days post hatch	nominal concentrations: 0, 0.063, 0.13, 0.25, 0.50, or 1.0 mg ai/L; mean measured concentrations: 0, 0.084, 0.14, 0.22, 0.36, or 0.71 mg ai/L by continuous exposure for 35 days. Both untreated and solvent controls were used.	The study and the supplement report no adverse effects on organism survival at hatch, larval survival and larval length and weight at any concentration levels. The U.S. EPA has classified the 0.71 mg/L concentration as an effect level based on decreased survival (88%) relative to survival in the solvent control (98%).	Bettancourt 1992 MRID 42436242 Surprenant 1994 MRID 43145701 (Supplement)

Appendix 8: Toxicity of tebufenozide to aquatic invertebrates and algae.

Plant or Animal	Exposure	Response	Reference

Aquatic Invertebrates

ACUTE

Plant or Animal	Exposure	Response	Reference
Cladoceran (*Daphnia magna*), neonates (<24-hours old), 2 replicates of 10 each per dose group	nominal test concentrations: 0, 0.25, 0.50, 1.0, 2.5, 5.0, 10, or 100 mg ai/L; mean measured concentrations: 0, 0.22, 0.50, 0.82, 1.8, 4.7, 6.4, or 35 mg ai/L for 48 hours under static conditions	48-hour LC_{50} = 3.8 mg ai/L (95% CI = 2.9 and 5.1 mg ai/L) NOEC = 0.82 mg ai/L no signs of toxicity at concentrations ≤0.82 mg ai/L; values >1.8 ai/L were considered to be above the functional water solubility of the test substance.	Graves and Smith 1992a MRID 42436241
Northern lobsters (*Homarus americanus*), juveniles, 50-80 mm long	1.0, 10, or 100 µg ai/L Confirm 2F for 96 hours under static conditions	No adverse effects on survival and behavior.	Dionne 1998 MRID 44945701
Midge larvae (*Chironomus riparius*), 20 larvae (2 replicates of 10 animal each)	0, 0.05, 0.1, 0.2, 0.4, or 0.8 mg ai/L for 96 hours under static conditions Both untreated and solvent controls (acetone 0.10 mL/L).	96-hour aqueous LC_{50} = 0.30 mg ai/L (95% CI = 0.23-0.40 mg ai/L 96-hour NOEC = 0.12 mg ai/L both values based on mean measured concentrations.	van der Kolk 1997 MRID 44198301

Appendix 8-1

Plant or Animal	Exposure	Response	Reference

Aquatic Invertebrates (continued)

LONGER-TERM

| *Daphnia magna*, 10 per replicate vessel | Continuous exposure to 16, 29, 59, 120, or 240 µg ai/L for 21 days under flow-through conditions. | **Mortality**: at 21 days, average mean survival at 240 µg ai/L group= 50%, significantly less (p<0.05), than controls (96%); survival in lower dose groups ranged from 93-100%. | McNamara 1991 MRID 42436243 |

Additional Notes on McNamara 1991:
Reproduction: at 120 µg ai/L, statistically significant decrease (p$\leq$0.05) in average rate of offspring/female (n=143), compared with controls (n=188); at lower concentrations, rate of offspring/females ranged from 226 to 239, which is statistically comparable to control.

Growth: at 120 µg ai/L, statistically significant decrease (p$\leq$0.05) in mean total body length (5.0 mm), compared with controls (5.4 mm); at lower concentrations, mean total body length ranged from 5.3 to 5.5, which is statistically comparable to controls;

at 59 and 120 µg ai/L, statistically significant decrease (p$\leq$0.05) in mean dry weight (1.3 and 1.6 mg, respectively), compared with controls (1.9 mg); at lower concentrations, mean dry weight ranged from 1.9 to 2.0, which is statistically comparable to controls;

LOEC = 59 µg ai/L; NOEL = 29 µg ai/L
21-day EC_{50} = 250 µg ai/L (lower 95% confidence interval of 120 µg ai/L)

| Midge larvae (*Chironomus riparius*), 2- to 3-days old, 4 replicates per dose group | 0, 0.0035, 0.0053, 0.0079, 0.012, 0.018, 0.027, 0.040, 0.060,0.090, or 0.135 mg ai/L for 28 days

Both untreated and solvent controls (acetone 0.10 mL/L). | No effect on development rate of midge at any concentration; at $\geq$0.040 no midge emerged, which precluded the calculation of a development rate; at 0.0053, there was a statistically significant (p$\leq$0.05) decrease in emergence rate; NOEC = 0.0035. | van der Kolk 1997 MRID 44198301 |

Appendix 8: Toxicity of tebufenozide to aquatic invertebrates and algae.

Plant or Animal	Exposure	Response	Reference
		Aquatic Algae	
Freshwater green alga (*Scenedesmus subspicatus*)	0.046, 0.077, 0.15, 0.25, or 0.66 mg ai/L (63-89% of nominal concentration) for 96 hours. Both untreated and solvent controls (acetone 0.10 mL/L).	**Cell density:** at 0.077, 0.15, 0.25, and 0.66 mg ai/L, respective cell densities averaged 81, 58, 52, and 37 x 10^4 cells/mL and were statistically reduced compared with pooled control cultures (114 x 10^4 cells/mL); at the lowest treatment level, cell density was statistically similar to that of controls). **Growth rate**: at 0.15, 0.25, and 0.66 mg ai/L, the 72-96 hr growth rates were 0.259, 0.310, and 0.004 days^{-1}, respectively and were statistically reduced compared with the growth rate of pooled controls (0.594 days^{-1}) NOEC for 72-96 hr growth rate = 0.077 mg ai/L. The 96 hr EC_{50} = 0.21 mg ai/L (95% confidence limit = 0.071-0.63 mg ai/L)	Hoberg 1992a MRID 42629501
Freshwater green alga (*Selenastrum capricornutum*) replicate 50 mL cultures (3 per treatment levels)	Nominal concentration of 0.80 mg ai/L for 120 hours	Empirically estimated EC_{50} >0.64 mg ai/L NOEC (based on reduced cell density) = 0.64 ai/L Treated algal culture reduced in density by 9.1% compared with controls	Hoberg. 1992b MRID 42436245 Reinert. 1993b MRID 42822201

Appendix K
DDVP (Dichlorvos)
Risk Assessment

Figure K-1. A sprayer unit mounted on a Model A Ford truck was used for gypsy moth control.

Control/Eradication Agents for the Gypsy Moth -
Human Health and Ecological Risk Assessment for
DDVP (Dichlorvos)
FINAL REPORT

Prepared for:

USDA, Forest Service
Forest Health Protection

GSA Contract No. **GS-10F-0082F**
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Task No. **5**

Submitted to:
Dave Thomas, COTR
Forest Health Protection Staff
USDA Forest Service
Rosslyn Plaza Building C, Room 7129C
1601 North Kent Street
Arlington, VA 22209

Prepared by:
Patrick R. Durkin and Mark H. Follansbee [a]
Syracuse Environmental Research Associates, Inc.
5100 Highbridge St., 42C
Fayetteville, New York 13066-0950
Telephone: (315) 637-9560
Fax: (315) 637-0445
E-Mail: SERA_INC@msn.com
Home Page: www.sera-inc.com

[a] Syracuse Research Corporation, 301 Plainfield Road, Suite 350, Syracuse, New York 13212

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Supplement 1: EXCEL Worksheets for Human Health and Ecological Risk Assessments of
DDVP. SERA EXWS 04-43-05-05b, dated August 18, 2004.

Located at: http://www.fs.fed.us/foresthealth/pesticide/risk.shtml

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AEL	adverse-effect level
ACGIH	American Conference of Governmental Industrial Hygienists
AChE	acetylcholinesterase
a.i.	active ingredient
BCF	bioconcentration factor
bw	body weight
CBI	confidential business information
ChE	pseudo-cholinesterase
CI	confidence interval
cm	centimeter
CNS	central nervous system
DAA	days after application
DAT	days after treatment
d.f.	degrees of freedom
EC_x	concentration causing X% inhibition of a process
EC_{25}	concentration causing 25% inhibition of a process
EC_{50}	concentration causing 50% inhibition of a process
ExToxNet	Extension Toxicology Network
F	female
FH	Forest Health
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FOIA	Freedom of Information Act
FQPA	Food Quality Protection Act
g	gram
ha	hectare
HQ	hazard quotient
IAA	indole-3-acetic acid
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
k_a	absorption coefficient
k_e	elimination coefficient
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
$K_{o/w}$	octanol-water partition coefficient
K_p	skin permeability coefficient
L	liter
lb	pound
LC_{50}	lethal concentration, 50% kill
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level

LOC	level of concern
m	meter
M	male
MCL	mononuclear cell carcinoma
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
mM	millimole
MRID	Master Record Identification Number
MSDS	material safety data sheet
MW	molecular weight
NCAP	Northwest Coalition for Alternatives to Pesticides
NCI	National Cancer Institute
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOS	not otherwise specified
NRC	National Research Council
NTP	National Toxicology Program
OM	organic matter
OPIDN	organophosphate-induced delayed neurotoxicity
OPP	Office of Pesticide Programs
OPPTS	Office of Pesticide Planning and Toxic Substances
OSHA	Occupational Safety and Health Administration
ppm	parts per million
PVC	polyvinyl chloride
RBC	red blood cell
RED	re-registration eligibility decision
RfD	reference dose
SERA	Syracuse Environmental Research Associates
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SRC	Syracuse Research Corporation
STS	Slow the Spread
UF	uncertainty factor
U.S.	United States
USDA	U.S. Department of Agriculture
U.S. EPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WCR	water contamination rate
WHO	World Health Organization
ì	micron

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

To convert ...	Into ...	Multiply by ...
acres	hectares (ha)	0.4047
acres	square meters (m^2)	4,047
atmospheres	millimeters of mercury	760
centigrade	Fahrenheit	1.8 °C+32
centimeters	inches	0.3937
cubic meters (m^3)	liters (L)	1,000
Fahrenheit	centigrade	0.556 °F-17.8
feet per second (ft/sec)	miles/hour (mi/hr)	0.6818
gallons (gal)	liters (L)	3.785
gallons per acre (gal/acre)	liters per hectare (L/ha)	9.34
grams (g)	ounces, (oz)	0.03527
grams (g)	pounds, (oz)	0.002205
hectares (ha)	acres	2.471
inches (in)	centimeters (cm)	2.540
kilograms (kg)	ounces, (oz)	35.274
kilograms (kg)	pounds, (lb)	2.2046
kilograms per hectare (hg/ha)	pounds per acre (lb/acre)	0.892
kilometers (km)	miles (mi)	0.6214
liters (L)	cubic centimeters (cm^3)	1,000
liters (L)	gallons (gal)	0.2642
liters (L)	ounces, fluid (oz)	33.814
miles (mi)	kilometers (km)	1.609
miles per hour (mi/hr)	cm/sec	44.70
milligrams (mg)	ounces (oz)	0.000035
meters (m)	feet	3.281
ounces (oz)	grams (g)	28.3495
ounces per acre (oz/acre)	grams per hectare (g/ha)	70.1
ounces per acre (oz/acre)	kilograms per hectare (kg/ha)	0.0701
ounces fluid	cubic centimeters (cm^3)	29.5735
pounds (lb)	grams (g)	453.6
pounds (lb)	kilograms (kg)	0.4536
pounds per acre (lb/acre)	kilograms per hectare (kg/ha)	1.121
pounds per acre (lb/acre)	mg/square meter (mg/m^2)	112.1
pounds per acre (lb/acre)	ì g/square centimeter (ì g/cm^2)	11.21
pounds per gallon (lb/gal)	grams per liter (g/L)	119.8
square centimeters (cm^2)	square inches (in^2)	0.155
square centimeters (cm^2)	square meters (m^2)	0.0001
square meters (m^2)	square centimeters (cm^2)	10,000
yards	meters	0.9144

Note: All references to pounds and ounces refer to avoirdupois weights unless otherwise specified.

CONVERSION OF SCIENTIFIC NOTATION

Scientific Notation	Decimal Equivalent	Verbal Expression
$1 \cdot 10^{-10}$	0.0000000001	One in ten billion
$1 \cdot 10^{-9}$	0.000000001	One in one billion
$1 \cdot 10^{-8}$	0.00000001	One in one hundred million
$1 \cdot 10^{-7}$	0.0000001	One in ten million
$1 \cdot 10^{-6}$	0.000001	One in one million
$1 \cdot 10^{-5}$	0.00001	One in one hundred thousand
$1 \cdot 10^{-4}$	0.0001	One in ten thousand
$1 \cdot 10^{-3}$	0.001	One in one thousand
$1 \cdot 10^{-2}$	0.01	One in one hundred
$1 \cdot 10^{-1}$	0.1	One in ten
$1 \cdot 10^{0}$	1	One
$1 \cdot 10^{1}$	10	Ten
$1 \cdot 10^{2}$	100	One hundred
$1 \cdot 10^{3}$	1,000	One thousand
$1 \cdot 10^{4}$	10,000	Ten thousand
$1 \cdot 10^{5}$	100,000	One hundred thousand
$1 \cdot 10^{6}$	1,000,000	One million
$1 \cdot 10^{7}$	10,000,000	Ten million
$1 \cdot 10^{8}$	100,000,000	One hundred million
$1 \cdot 10^{9}$	1,000,000,000	One billion
$1 \cdot 10^{10}$	10,000,000,000	Ten billion

EXECUTIVE SUMMARY

OVERVIEW

The USDA uses DDVP in its program to manage the gypsy moth. The primary use of DDVP is as a component in the pheromone baited milk carton style traps that are used primarily for surveying and monitoring gypsy moth populations. Because of this a very limited use in USDA programs, the potential for exposures to humans or nontarget ecological species is extremely limited. Because of this limited use and limited potential for exposure, this risk assessment focuses on the information that has the greatest impact on potential hazard rather than a summary of all of the available information that is available on DDVP and this risk assessment utilizes several detailed reviews conducted by agencies responsible for assessing chemical risks

PROGRAM DESCRIPTION

In USDA programs for the control of the gypsy moth, DDVP is used only in a 1" x 4" inch polyvinyl chloride (PVC) strip that contains 590 mg of DDVP. These strips are used to kill insects that are attracted to and enter milk carton style traps baited with the gypsy moth pheromone. Typically milk carton traps are deployed in widely spaced grids (inter-trap distances ranging from 500 m to 7 km) to survey for the presence of gypsy moth populations in the STS or eradication areas. Only rarely are milk carton traps deployed in mass trapping grids to control isolated infestations. When used in mass trapping control efforts, milk carton traps are deployed in tightly spaced grids (inter-trap distance of 20 to 30 meters). Mass trapping is a rarely used eradication tactic that targets low-density infestations (<10 egg masses per acre) occupying relatively small areas (<100 acres) .

HUMAN HEALTH RISK ASSESSMENT

Hazard Identification – DDVP is an organophosphorus insecticide that works by inhibiting cholinesterase. DDVP has been used since the early 1960's and has been the subject of many toxicity studies and review articles. Information is available on a number of case reports of accidental and suicidal exposures as well as human monitoring data from normal use. The toxicity of DDVP has been adequately evaluated using laboratory animals, although not all of these studies are available in the open literature.

DDVP is readily absorbed into the body of mammals via all routes of exposure, where it is rapidly metabolized and eliminated. In general, the systemic effects observed after oral, inhalation, or dermal exposure of humans or laboratory animals to DDVP result from the inhibition of acetylcholinesterase. Inhibition of this enzyme in mammalian systems produces a variety of systemic effects, including salivation, urination, lacrimation, convulsions, increased bronchial secretions, respiratory depression, and even death. The nature and magnitude of the toxic effects produced by a given exposure to DDVP by any route are directly related to the dose and rate at which the exposure occurs. In the case of the USDA programs for the management of the gypsy moth, the use of milk carton traps containing Vaportape II (slow-release of DDVP from PVC strips) essentially precludes rapid exposures to high doses of DDVP.

Short-term animal studies have shown that oral exposures to doses below about 0.5 mg/kg-day (or inhalation exposures to 1–2 mg/m³) do not result in meaningful reductions in cholinesterase activity. Experiments in laboratory mammals that were exposed to DDVP during pregnancy (by oral or inhalation route) did not show any effect on fertility or health of the offspring, even at levels that produced maternal toxicity. The latest evaluation of data from assays for carcinogenicity and genetic toxicity classify DDVP as a "suggestive" carcinogen and determined that a quantitative assessment of cancer risk is not applicable. The literature contains some data suggesting that contact dermatitis (as well as cross-sensitization to other pesticides) may occur; although, this appears to be an infrequent occurrence in the general population.

Exposure Assessment – Under normal conditions, exposure to both workers and members of the general public should be negligible. Workers will handle DDVP strips only during the assembly of milk cartoon traps. If workers wear gloves and assemble the traps outdoors or in very well ventilated rooms, both inhalation and dermal exposures should be negligible. Inhalation exposure to DDVP during transport of the traps should also be negligible if the traps are not transported inside of the passenger compartments of vehicles. Worker exposures will also be limited in most programs because foil wrapping in which the strip is distributed will not be removed until after the trap is transported to the field. Milk carton traps will generally be placed about four feet above the ground (Leonard 2004) and exposure of members of the general public to DDVP contained in the milk carton traps should also be negligible except in the case of intensional tampering.

Notwithstanding the above assertions, exposure assessments are developed for workers who do not use gloves in the assembly of the milk carton traps and who assemble the traps indoors and transport the traps in the passenger compartments of vehicles. All of these exposure scenarios should be considered atypical and some are extreme. The intent is to illustrate the consequences of mishandling or imprudent handling. During assembly, the central estimate of dermal exposures in workers not wearing gloves leads to an absorbed dose of about 0.0008 mg/kg with a range of about 0.0003 mg/kg to 0.004 mg/kg. Inhalation exposures in workers may be highly variable depending on the ventilation rates in an enclosed space and the number of traps that are handled. Based on the handling and transport of 75 traps, inhalation exposures could reach up to about 0.6 mg/m^3 in an enclosed and unventilated room and up to about 1.8 mg/m^3 in the passenger compartment of a vehicle. These exposure assessments are based on several site and situation specific assumptions which are intended to reflect plausible upper bounds of exposures.

Exposure assessments are also developed for children who might come in contact with an accidentally discarded or misplaced DDVP strip. Estimated dermal doses are much higher than those for workers: a central estimate of about 0.02 mg/kg with a range of 0.003 mg/kg to 0.1 mg/kg. Oral exposures from a small child sucking on the pest strip are about a factor of 10 higher than dermal exposures: a central value of about 0.2 mg/kg with a range of 0.04 mg/kg to 0.6 mg/kg.

Under normal circumstances, the use of DDVP in PVC strips is not likely to result in contamination of water or other materials that might be consumed by members of the general public. Nonetheless, an exposure assessment is developed for the accidental contamination of a small pond by a pest strip. In this scenario, dose estimates range from about 0.000003 mg/kg to 0.00007 mg/kg with a central estimate of about 0.00001 mg/kg.

Dose-Response Assessment – The extensive toxicology data base has been evaluated by a number of governmental organizations including the U.S. EPA, the Agency for Toxic Substances and Disease Registry (ATSDR), National Institute for Occupational Safety and Health (NIOSH), Occupational Safety and Health Administration, and the World Health Organization. Following the approach taken in most USDA risk assessments, these sources are used for selecting levels of acceptable exposure. Because all of the scenarios considered in this risk assessment involve only acute exposures, only acute exposure criteria are considered.

For both oral and dermal exposures, the acute RfD established by the U.S. EPA, 0.0017 mg/kg, is used for the risk characterization. This is based on an acute oral NOAEL of 0.5 mg/kg from a study in rats with the application of an uncertainty factor of 300. Acute exposure criteria proposed by other groups are comparable to but somewhat higher than the acute RfD. Because some of the accidental acute exposures may substantially exceed the acute RfD, some attempt is made to characterize the consequences of high oral exposures. A human NOAEL of 1 mg/kg for AChE inhibition has been identified. While this NOAEL is not used to modify the acute RfD, it can be used to assess plausible consequences of exceeding the RfD. The human data on DDVP, although extensive, are not sufficient to identify a minimal lethal dose. For the current risk assessment, the lowest reported lethal dose (16 mg/kg) is used to assess the plausibility of observing serious adverse effects in cases of accidental over-exposure to DDVP.

A number of inhalation criteria for DDVP are available. Since potentially significant inhalation exposures are likely only in workers, the occupational exposure criteria of 0.1 mg/m³ proposed by American Conference of Governmental Industrial Hygienists is used. This value is a factor of 10 below the occupational criteria proposed by NIOSH and OSHA.

Risk Characterization – In most cases, exposures to both workers and members of the general public should be negligible. If workers take prudent steps to limit both dermal and inhalation exposures, the likelihood of exposures to DDVP reaching a level of concern appears to be very low. Similarly, members of the general public should not be exposed to substantial amounts of DDVP. The DDVP is contained within a PVC strip to insure that the active ingredient is slowly released over a long period of time. The strip, in turn, is placed within a trap and the trap is placed so that the that will not be accessed except in the case of intentional tampering or trap monitoring.

Nonetheless, this risk assessment develops exposure scenarios for both workers and members of the general pubic that are intended to illustrate the potential effects of mishandling or tampering with DDVP strips. For workers, the greatest risks are associated with inhalation exposures from

assembling the traps in enclosed and poorly ventilated spaces or transporting the traps in the passenger compartments of vehicles. These risks can be readily avoided. Dermal exposures can also lead to lesser but sill undesirable levels of exposure. For members of the general public, all of the exposure scenarios are accidental and some are extreme. The most likely of these is the accidental contamination of a small body of water. This scenario leads to exposures that are below the level of concern by a factor of about 25. If a child were to come into contact with a DDVP strip, however, both dermal and oral exposures could substantially exceed a level of concern. While such exposures should clearly be avoided, it does not seem likely that frank signs of toxicity would be observed. This is consistent with human experience in the use of DDVP resin strips.

ECOLOGICAL RISK ASSESSMENT

Hazard Identification – The available data suggest that invertebrates are more sensitive to DDVP than other organisms. For example, the oral LD_{50} in honey bees is 0.29 ì g/g bee, and the topical LD_{50} is 0.65 ì g/g bee. DDVP is also toxic to birds with an oral LD_{50} value of < 10 mg/kg for the most sensitive species. Short-term repeat dose studies in mammals found that oral exposures to doses below about 0.5 mg/kg-day or inhalation exposures to 1–2 mg/m³ generally do not result in adverse effects.

Aquatic animals are also sensitive to DDVP and, as with terrestrial animals, invertebrates may be more sensitive than vertebrates. The lowest reported LC_{50} value in fish is approximately 0.2 mg/L. Some aquatic invertebrates are much more sensitive to DDVP than fish. For daphnids, the most sensitive group of invertebrate species, reported EC_{50} values range from 0.00007 mg/L to 0.00028 mg/L.

The majority of the toxicity data in ecological receptors is limited to free DDVP, rather than a slow-release formulation such as the Vaportape II product used in USDA programs for control of the gypsy moth. Hence, the toxicity values reported for indicator species will likely be conservative (i.e., suggest greater toxicity) as compared to Vaportape II. U.S. EPA has assessed the ecological effects of DDVP; however, the exposures assessed by U.S. EPA are not specific to formulations where DDVP is encapsulated in PVC resin. In general, aside from those organisms that enter the milk carton trap or those that remove the PVC strip form the trap, toxicity resulting from exposure of ecological receptors to DDVP in Vaportape II milk carton traps is not likely.

Exposure Assessment – As in the human health risk assessment, exposure of terrestrial mammals to DDVP from the VaporTape strips used in milk carton traps is likely to be negligible under most circumstances. Nonetheless, it is conceivable that some mammals such as racoons or bears could easily access and tamper with the milk carton trap. Depending on the proportion of the DDVP strip that is consumed, doses (as DDVP in the PVC strip) are estimated to range from 10.5 mg/kg (10% of strip) to 105 mg/kg (100% of strip) and the central estimate is taken as 31.6 mg/kg (30% of strip). In addition, contamination of water with a pest strip is plausible, although probably rare, and is considered in a manner similar to the corresponding scenario in the human health risk assessment (Section 3.2.3.4). This scenario is based on the consumption of

contaminated water by a small mammal and the dose to the animal is estimated at about 0.00003 mg/kg with a range of 0.000009 mg/kg to 0.00009 mg/kg. Other exposure scenarios for terrestrial vertebrates, while possible, seem far less plausible and are not considered quantitatively. No quantitative exposure assessments for terrestrial invertebrates are developed because the milk carton trap will attract only male gypsy moths. Nontarget insects that incidentally enter the trap are likely to be killed by exposure to the DDVP vapor. Exposures to aquatic species are based on the same water concentrations used for terrestrial species: 0.000177 mg/L with a range of 0.000059 mg/L to 0.00059 mg/L.

Dose-Response Assessment – Given the limited nature of the use of DDVP in programs to control the gypsy moth and consequent limited number of exposure assessments, the dose-response assessment for DDVP is relatively simple. For terrestrial mammals, a value of 240 mg/kg from a study using DDVP in a PVC formulation is used for direct exposure to the DDVP-PVC strip – i.e., a raccoon tampering with a milk carton trap and consuming all or part of the DDVP strip. At the dose of 240 mg/kg, no mortality or frank signs of AChE inhibition were observed. For the contaminated water scenario, the NOAEL of 0.5 mg/kg from a study involving exposure to free or unformulated DDVP is used. This NOAEL is from the study that forms the basis for the acute RfD used in the human health risk assessment. Although DDVP is classified as highly toxic to fish, the estimated levels of acute exposure for fish are far below the 30-day NOEC of 0.03 mg/L. Thus, this value is used for all fish and no attempt is made to consider differences in sensitivity among fish. A somewhat different approach is taken with aquatic invertebrates, some of which are more sensitive to DDVP than fish by a factor of over 2500. Risks to sensitive species of aquatic invertebrates – i.e., daphnids and other small arthropods – are characterized based on the lowest reported LC_{50} value, 0.00007 mg/L from a 48-hour bioassay in *Daphnia pulex*. Some other groups of aquatic invertebrates, such as snails, appear to be much less sensitive than small arthropods. Risks to such tolerant species are based on a LC_{50} value of 21 mg/L in a freshwater snail.

Risk Characterization – As with the human health risk assessment, it is anticipated that typical exposures and consequent risks to nontarget species should be negligible. As with the human health risk assessment, it is anticipated that typical exposures and consequent risks to most nontarget species should be negligible. The containment of the DDVP within a slow release PVC strip combined with the target specific nature of pheromone baited traps should reduce the risks of inadvertent effects in non-target species. Other insects and arthropods that may inadvertently enter the trap will probably be killed by DDVP vapor. While such inadvertent contact may occur, it is not likely to impact substantial numbers of nontarget insects or arthropods.

Because of the limited use of DDVP, a relatively small number of exposure scenarios – all of which might be considered accidental or incidental – are developed. For terrestrial mammals, contact with the pest strip could occur by an animal directly tampering with a trap or by an animal consuming water that had been accidentally contaminated with a DDVP strip. Adverse effects would not be expected in either case. In the case of accidental contamination of a small

body of water with a DDVP strip, concentrations of DDVP in the water would be below the level of concern for fish by factors of about 50 to 500. Some aquatic invertebrates, however, might be affected. For the most sensitive species of aquatic invertebrates – i.e., small aquatic arthropods such as daphnids – exposures could substantially exceed laboratory LC_{50} values by factors of up to about 8. Exposures to tolerant aquatic invertebrates – such as snails – would be below a level of concern by a substantial margin – i.e., factors of about 30,000 to 300,000.

The exposure assessments that serve as the bases for these risk characterizations are highly dependent on specific conditions – i.e., how much DDVP was in the strip at the time that the contamination occurred and the size of the body of water that was contaminated. Because the hydrolysis of DDVP in water is rapid, the estimates of adverse effects in some aquatic invertebrates would probably apply only to a very limited area near the pest strip rather than to the larger area of the body of water that is contaminated.

1. INTRODUCTION

The USDA uses DDVP in its program to manage the gypsy moth. The primary use of DDVP is as a component in the pheromone baited milk carton style traps that are used primarily for surveying and monitoring gypsy moth populations. This document is an update to a risk assessment prepared in 1995 (USDA 1995a,b) and provides risk assessments for human-health effects and ecological effects to support an assessment of the environmental consequences of these uses.

This document has four chapters, including the introduction, program description, risk assessment for human health effects, and risk assessment for ecological effects or effects on wildlife species. Each of the two risk assessment chapters has four major sections, including an identification of the hazards associated with DDVP, an assessment of potential exposure to the product, an assessment of the dose-response relationships, and a characterization of the risks associated with plausible levels of exposure. These are the basic steps recommended by the National Research Council of the National Academy of Sciences (NRC 1983) for conducting and organizing risk assessments.

Although this is a technical support document and addresses some specialized technical areas, an effort was made to ensure that the document can be understood by individuals who do not have specialized training in the chemical and biological sciences. Certain technical concepts, methods, and terms common to all parts of the risk assessment are described in plain language in a separate document (SERA 2001).

The human health and ecological risk assessments presented in this document are not, and are not intended to be, comprehensive summaries of all of the available information. This is particularly true for DDVP used in gypsy moth programs. There is an extremely large and relatively complex database of literature on DDVP. For example, TOXLINE, one of several commonly used commercial databases containing information on toxic chemicals, has over 14,000 citations on DDVP. DDVP, however, has a very limited use in USDA gypsy moth programs (Section 2) and the potential for exposures to humans (Section 3.2) or nontarget ecological species (Section 4.2) is extremely limited. Because of this limited use and limited potential for exposure, this risk assessment focuses on the information that has the greatest impact on potential hazard rather than a summary of all of the available information that is available on DDVP and this risk assessment utilizes several detailed reviews conducted by agencies responsible for assessing chemical risks (e.g., ATSDR 1997; U.S. EPA 1999a, 2000a,b; WHO 1988, 1989).

This risk assessment involves numerous calculations. Many of the calculations are relatively simple and the very simple calculations are included in the body of the document. Some of the calculations, however, are complex. For the more complex calculations, worksheets are included as an attachment to the risk assessment. The worksheets provide the detail for the estimates cited in the body of the document. The worksheets for DDVP are contained in an EXCEL workbook

and are included as Supplement 1 to this risk assessment and general documentation for the use of these worksheets is given in SERA (2004).

The USDA will update this and other similar risk assessments on a periodic basis and welcomes input from the general public on the selection of studies included in the risk assessment. This input is helpful, however, only if recommendations for including additional studies specify why and/or how the new or not previously included information would be likely to alter the conclusions reached in the risk assessments.

2. PROGRAM DESCRIPTION

2.1. OVERVIEW

DDVP is an organophosphate insecticide that acts by inhibiting acetylcholinesterase, an enzyme that is very important in the nervous system of all vertebrates and many invertebrates including all arthropods. Thus, DDVP is not specific to the gypsy moth or other insects. In USDA programs for the control of the gypsy moth, DDVP is used only in a 1" x 4" inch polyvinyl chloride (PVC) strip that contains 590 mg of DDVP. These strips are used to kill insects that are attracted to and enter milk carton style traps baited with the gypsy moth pheromone. Typically milk carton traps are deployed in widely spaced grids (inter-trap distances ranging from 500 m to 7 km) to survey for the presence of gypsy moth populations in the STS or eradication areas. Only rarely are milk carton traps deployed in mass trapping grids to control isolated infestations. When used in mass trapping control efforts, milk carton traps are deployed in tightly spaced grids (inter-trap distance of 20 to 30 meters). Mass trapping is a rarely used eradication tactic that targets low-density infestations (<10 egg masses per acre) occupying relatively small areas (<100 acres) .

2.2. CHEMICAL DESCRIPTION AND COMMERCIAL FORMULATIONS

DDVP is the common name for O,O-dimethyl O-(2,2-dichlorovinyl) phosphate:

Other synonyms for DDVP as well as selected chemical and physical properties of DDVP are summarized in Table 2-1.

DDVP is a contact and stomach organophosphate insecticide (Gallo and Lawryk 1991, IARC 1991). As detailed further in the human health risk assessment (Section 3) and the ecological risk assessment (Section 4), DDVP acts by inhibiting acetylcholinesterase, an enzyme that is very important in the nervous system of all vertebrates (including humans) and most other animals including all arthropods.

DDVP is currently undergoing reregistration (http://www.epa.gov/pesticides/op/ddvp.htm; Mennear 1998) and is being considered in the U.S. EPA's cumulative risk assessment of organophosphates (http://www.eps.gov/pesticides).

Various DDVP pest strips for residential or industrial use have been registered with the U.S. EPA and are manufactured by AMVAC Chemical Corporation, Loveland Industries, and Spectrum Group (http://www.cdpr.ca.gov/docs/pressrls/ddvp.htm). However, the only strip used by the USDA in gypsy moth programs is the Vaportape II strip provided by Hercon Environmental Corp, Emigsville, PA (Hercon 1993). A contract for the supply of these strips to

the USDA gypsy moth program was awarded to Hercon Environmental Corp on March 23, 1999 (www.fbodaily.com/cbd/archive/1999/03 (March)23/Mar-1999/87awdoo1.htm).

Vaportape II is distributed in packages of 50 strips, each of which comes in a protective pouch. Each strip consists of a 1" x 4" inch red, multi-layered polyvinyl chloride (PVC) strip containing 590 mg of DDVP. The average thickness of the strip is 67.5 mil with a range of 65–70 mil or 0.0675 inches with a range of 0.065–0.07 inches (Hercon 1994). Additional details concerning the composition of the strips have been disclosed to U.S. EPA (Health-Chem Corporation 19??; Herculite Products Incorporated 19??a,b; Starner 1993). Note that the 19?? designation indicates that the material is not dated and that the U.S. EPA cannot determine when the information was submitted. This is not uncommon for submissions that occurred in the early 1970's. The details of the information contained in these submissions are classified as CBI (confidential business Information) under Section 7(d) and Section (10) of FIFRA and this information cannot be specifically disclosed in this risk assessment.

The product label specifies that in addition to DDVP, each strip contains 0.75% compounds that are related to DDVP and 89.25% inerts (Hercon 2004). Further details are not provided on the label; nonetheless the impurities in commercial DDVP have been characterized (Gillett and others 1972a, IARC 1991). The impurities include: Dipterex (O,O-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate); O,O-dimethyl 2-chlorovinyl phosphate; O,O-dimethyl methylphosphonate; O,O,O-trimethyl phosphate; and trichloroacetaldehyde. These impurities are known to be or are likely to be toxic (Gillett and others 1972a, WHO 1989). These impurities are encompassed in the risk assessment because the dose-response assessment is based on studies that used commercial grade DDVP. Consequently, the results of these studies are directly applicable to the risk assessment for human health (Section 3) and ecological effects (Section 4).

2.3. APPLICATION METHODS

The Vaportape II strips are used as an insecticide in large capacity pheromone traps to monitor gypsy moth populations. DDVP is also used in a similar way in monitoring populations of the beet armyworm (Lopez 1998).

In order to minimize the ecological effects and human health effects of gypsy moth infestations, the USDA adopted various intervention strategies that are roughly categorized as suppression, eradication, and Slow the Spread (STS). Suppression efforts are conducted by the USDA Forest Service in areas of well established gypsy moth infestations to combat or interdict periodic gypsy moth population outbreaks. Eradication efforts are conducted by USDA/APHIS to completely eliminate gypsy moth populations in areas where new populations of the gypsy moth are found. Slow the spread, as the name implies, is a program to reduce the expansion of gypsy moth populations from areas of established populations to adjacent non-infested areas (Liebhold and McManus 1999). The STS project is the primary user of DDVP and milk carton traps. STS has purchased DDVP in the following amounts: 2002 - 540 packs (540x50 strips=27,000 strips); 2003 - 540 packs (27,000 strips); 2004 - 500 packs (25,000 strips) (Leonard 2004).

As in the previous gypsy moth programs, a Vaportape II strip is contained in the milk carton trap together with together with a slow release dispenser containing disparlure, the gypsy moth pheromone. The milk carton traps containing the strips are placed in selected areas to monitor gypsy moth infestations. When used in eradication efforts for mass trapping, milk carton traps are typically used only in low density infestations – i.e., 10 egg masses per acre or less. In addition, because of the labor involved in mass trapping, this method is applied to relatively small areas – i.e., about 100 acres or less (USDA 2001, p. 1-7 to 1-8).

As discussed in the exposure assessments for human health (Section 3.2) and ecological effects (Section 4.2), the nature of the exposures to humans and other nontarget species will typically be extremely small and it is unlikely that significant exposures will occur under normal circumstances. For workers, the nature of exposure to DDVP depends on program handling practices, which vary from state to state. In most cases, dermal and inhalation exposure will be minimal, provided that recommended work practices are followed. In some states, inhalation exposure will be minimal because strip installation takes place outdoors, at the trap placement site. In other states, traps may be assembled the day before placement. Even so, the workers are instructed to assemble the traps only in a well-ventilated area, and the traps are sealed in plastic bags after assembly and prior to transport. Dermal exposure is also likely to be minimal. In most states, workers are given plastic gloves and instructed to use them. In other states, workers are instructed to touch only the plastic wrapper in which the strip is shipped.

3. HUMAN HEALTH RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION
3.1.1. Overview
DDVP is an organophosphorus insecticide that works by inhibiting cholinesterase. DDVP has been used since the early 1960's and has been the subject of many toxicity studies and review articles. Information is available on a number of case reports of accidental and suicidal exposures as well as human monitoring data from normal use. The toxicity of DDVP has been adequately evaluated using laboratory animals, although not all of these studies are available in the open literature.

DDVP is readily absorbed into the body of mammals via all routes of exposure, where it is rapidly metabolized and eliminated. In general, the systemic effects observed after oral, inhalation, or dermal exposure of humans or laboratory animals to DDVP result from the inhibition of acetylcholinesterase. Inhibition of this enzyme in mammalian systems produces a variety of systemic effects, including salivation, urination, lacrimation, convulsions, increased bronchial secretions, respiratory depression, and even death. The nature and magnitude of the toxic effects produced by a given exposure to DDVP by any route are directly related to the dose and rate at which the exposure occurs. In the case of the USDA programs for the control of the gypsy moth, the use of milk carton traps containing Vaportape II (slow-release of DDVP from PVC strips) essentially precludes rapid exposures to high doses of DDVP.

Short-term animal studies have shown that oral exposures to doses below about 0.5 mg/kg-day (or inhalation exposures to 1–2 mg/m³) do not result in meaningful reductions in cholinesterase activity. Experiments in laboratory mammals that were exposed to DDVP during pregnancy (by oral or inhalation route) did not show any effect on fertility or health of the offspring, even at levels that produced maternal toxicity. The latest evaluation of data from assays for carcinogenicity and genetic toxicity classify DDVP as a "suggestive" carcinogen and determined that a quantitative assessment of cancer risk is not applicable. The literature contains some data suggesting that contact dermatitis (as well as cross-sensitization to other pesticides) may occur; although, this appears to be an infrequent occurrence in the general population.

3.1.2. Mechanism of Action
The mechanism of action of DDVP in target organisms and its principal toxic effects in humans and animals result from inhibiting neural acetylcholinesterase (AChE). DDVP shares this mechanism of action with other organophosphate insecticides. A number of excellent reviews on the mechanism of action of the organophosphate insecticides are available in various texts (Wills 1972; Gallo and Lawryk 1991; Taylor 1996; Ecobichon 2001). The AChE enzyme is present at cholinergic synapses (spaces between the nerve cells) throughout the nervous systems, and it is responsible for hydrolyzing acetylcholine released from the pre-synaptic terminal. If this enzyme is inhibited, acetylcholine accumulates in the synapse, resulting in increased stimulation of the postsynaptic neuron and cholinergic overstimulation. The consequences of increased cholinergic activity in various organ systems are listed in Table 3-1. These classical symptoms of

organophosphate neurotoxicity increase in severity and rapidity of onset in a dose-dependent manner.

Acetylcholinesterase is also present in erythrocytes where it is known as erythrocyte or red blood cell acetylcholinesterase (RBC AChE). *In vitro* assays have found that the erythrocyte and neural forms of AChE are inhibited to roughly the same extent by exposure to DDVP (ATSDR 1997). Measurement of RBC AChE is used as a surrogate of the inhibition of neural AChE. One of the major diagnostic tools and measures of exposure to DDVP and other organophosphate insecticides is the determination of cholinesterase activity in various tissues, most often red blood cells and plasma (Ecobichon 2001; Gallo and Lawryk 1991; Murphy 1980). Plasma cholinesterase, sometimes referred to as pseudo-cholinesterase or ChE, is produced by the liver and differs from AChE in structure and substrates (ATSDR 1993). Although the normal physiological role of plasma ChE is not known, it is also inhibited by DDVP and is often used as a marker for exposure. Inhibition of RBC AChE is generally regarded as a more clinically significant index of organophosphate exposure, compared with inhibition of plasma ChE, as plasma ChE is inhibited by DDVP at lower levels of exposure than required to inhibit neural or erythrocyte AChE (ATSDR 1997).

3.1.3. Kinetics and Metabolism

DDVP is a small, lipid-soluble molecule (see Table 2-1) that is readily absorbed by passive diffusion through the lungs, gastrointestinal tract, or skin. Little information is available on the pulmonary absorption rate of DDVP, but it appears to be rapidly absorbed by the inhalation as well as oral and dermal routes of exposure. Due to the rapid degradation of DDVP by tissue esterases, particularly in the liver and the serum, measuring DDVP *in vivo* is difficult. Laws (1966) reported that DDVP is absorbed primarily by hepatic portal venous system after oral administration and is subject to first pass metabolism by the liver. Because of the difficulty in measuring DDVP *in vivo*, the rate of absorption is typically inferred from the time to onset of clinical signs of AChE inhibition (see Table 3-1). Determination of the tissue distribution of DDVP is also difficult to study because of rapid metabolism, but the data do not suggest preferential distribution or sequestration in any tissue (ATSDR 1997). A compartmental model has been proposed by Garcia-Repetto et al. (1995) to describe the toxicokinetics of DDVP following oral exposure. The model was composed of two compartments: central and peripheral. The central compartment was blood, and the peripheral compartment encompassed adipose, muscle, and liver.

3.1.3.1. Oral Absorption – Oral absorption of DDVP is rapid. Acute oral toxicity studies have demonstrated toxic effects from oral DDVP exposure within minutes. ATSDR (1997) noted that animal studies demonstrated lethality from single gavage doses of DDVP within 9 minutes for Swiss mice and 15–30 minutes in crossbred swine; signs of cholinergic toxicity (vomiting and diarrhea) were noted in greyhound dogs 7–15 minutes after receiving oral doses of DDVP in gelatin capsules. Based on a suicide case, Shimizu et al. (1996) have reported the tissue distribution of DDVP in humans following oral exposure. Tissue to blood ratios in this individual ranged from <1 for brain and liver to 28 for heart and 115 for the spleen. The authors

reported that the high-tissue concentrations in the heart and spleen were likely due to diffusion from the stomach to nearby organs (postmortem, the stomach contained approximately 250 mL of fluid equivalent to 300 g of DDVP). Studies in swine treated with DDVP-impregnated PVC pellets (veterinary use as anthelminthic) show that DDVP is absorbed from the PVC resin after oral exposure (Jacobs 1968, Potter et al. 1973).

3.1.3.2. Dermal Absorption – No studies have been found on the dermal absorption rate of DDVP in humans. As a small, lipid-soluble compound (see Section 2.2), DDVP would likely be rapidly absorbed through the skin. Dermal absorption in rats has been studied by Jeffcoat (1990). Groups of rats were dosed with ^{14}C-DDVP at 3.6, 36, and 360 µg/rat by applying the compound to the shaved back. The treated area was isolated with a protective cover for a 10-hour period. After 10 hours, the remaining DDVP was washed from the treated surface and animals were sacrificed over 24- to 102-hour periods. Based on the ^{14}C recovered from the rats, the amount penetrating the skin ranged from 21.9 to 30.1% with no substantial variation among dose groups. For this type of a study, first order dermal absorption coefficients (*k*) can be calculated as:

$$k = -\ln(1-f)/t$$

where *f* is the fraction absorbed and *t* is the duration of exposure. Based on absorption fractions of 0.219 to 0.301, the first-order dermal absorption rates can be calculated as 0.025 hour^{-1} [-ln(1-0.219)/10 hours] to 0.036 hour^{-1} [-ln(1-0.301)/10 hours]. These calculations are based on the cumulative amount of DDVP recovered from urine, feces, expired air, blood, carcass, and treated skin). Excluding treated skin, only 6.4 to 11.4% of the dose was actually absorbed. These correspond to first order dermal absorption rates of 0.0066 hour^{-1} [-ln(1-0.064)/10 hours] to 0.012 hour^{-1} [-ln(1-0.114)/10 hours] and these estimates are consistent with the dermal absorption rate selected by EPA (2000a) for occupational and residential exposures (11% in 10 hours of exposure).

3.1.3.3. Metabolism – As noted above, DDVP is rapidly degraded by tissue esterases, particularly in the liver and the serum. The products of the esterase-catalyzed degradation of DDVP are dimethyl phosphate and dichloroacetaldehyde. Dimethyl phosphate is excreted in the urine, while dichloroacetaldehyde can be reduced to dichloroethanol or dehalogenated to glyoxal, which enters 2-carbon metabolism. Dichloroethanol is either conjugated to glucuronic acid and excreted in the urine or dehalogenated and further metabolized. There is also evidence that DDVP can be demethylated in a glutathione-dependent reaction (WHO 1989, ATSDR 1997). The *in vitro* half-life of DDVP in human blood is about 10 minutes (Blair et al. 1975).

3.1.4. Acute Oral Toxicity

As described in Section 3.1.2, DDVP exposure can result in increased cholinergic activity in the nervous system, producing the classical symptoms of organophosphate poisoning (See Table 3-1). The life-threatening effects of acute exposure to DDVP are usually related to its cholinergic effects on the respiratory system (respiratory depression, bronchospasm, increased bronchial secretions, pulmonary edema, and muscle weakness). DDVP is moderately to highly

toxic by the oral route when administered in single doses to a variety of animal species, and several cases of acute DDVP poisoning in humans have reported in the literature. Some individuals have committed suicide by intentionally ingesting DDVP pesticide formulations (e.g., Shimizu et al. 1996). This study is discussed further in Section 3.3 (Dose-Response Assessment). In an attempted suicide, a 56-year old woman who ingested about 100 mg/kg DDVP survived following intensive care for 14 days (WHO 1989). Two workers who had skin exposure to a concentrated dichlorvos formulation, and failed to wash it off, died of poisoning. In addition, four patients suffering from severe poisoning from oral exposure to dichlorvos survived, although they later showed delayed neurotoxic effects (WHO 1989). Thus, although the possibility of neuropathy in humans cannot be excluded, it is likely to occur only after almost lethal oral doses (see also Section 3.1.6).

Oral LD_{50} values for experimental mammals range from 25 to 300 mg/kg (Jones et al. 1968, Gaines 1969, Muller 1970, Wagner and Johnson 1970). Signs of intoxication in these studies are consistent with cholinergic overstimulation, typically salivation, lacrimation, urination, defecation, tremors, convulsions, and death from respiratory failure.

EPA (2000a, p. 18) identified an unpublished neurotoxicity study in rats as the basis for establishing a risk level for acute oral exposure to unformulated DDVP – i.e., DDVP not in a PVC strip. In this study (Bast et al. 1997), Sprague Dawley rats (12/sex/dose) received a single oral dose of DDVP (97.8%) at doses of 0, 0.5, 35, or 70 mg/kg. Behavioral testing, including a functional observation battery and motor activity, was conducted pretest, 15 minutes after treatment, and on days 7 and 14 after exposure. Cholinesterase activity was not measured in any tissue. The acute NOAEL was 0.5 mg/kg and the LOAEL was 35 mg/kg based on neurological effects related to AChE inhibition.

The containment of DDVP in a slow-release vehicle, however, such as the PVC in the Vaportape II strips, will reduce the likelihood of acute toxic effects. The kinetics of DDVP release from PVC were investigated in a study in which DDVP was incorporated into PVC at 20% (w/w) (Slomka and Hine 1981). The PVC was extruded, cut into pellets, and encased in a hard gelatin capsule. The release of DDVP from the capsules was assayed *in vitro* using an artificial gastric fluid and *in vivo* in swine and humans. The release rates in the three assays were comparable; approximately 30% was released in the first 24 hours, and the subsequent release appeared to follow a first order function with a release rate of approximately 0.1 day^{-1}.

The effect of PVC encapsulation on the toxicity of DDVP has been quantified in parallel acute assays in young pigs (Stanton et al. 1979) using unformulated DDVP (undiluted technical grade administered in gelatin capsules) and DDVP in PVC resin (administered by gavage). For the technical grade liquid formulation, the LD_{50} was 157 (113–227) mg/kg. Signs of toxicity in these animals were consistent with the general signs of AChE inhibition (Table 3-1) and included decreased general activity, vomiting, poor coordination, and twitching. In the bioassay using the PVC formulation, no deaths occurred at any of the administered doses – i.e., 180 mg/kg, 240 mg/kg, 320 mg/kg or 1,000 mg/kg. Higher doses of the DDVP-PVC formulation could not be

administered because these doses produced vomiting. While not specified by Stanton et al. (1979), vomiting at doses >1,000 mg/kg may have been due to the physical stress associated with such a large gavage dose. Although no animals died, vomiting was observed at all DDVP-PVC doses. At the lowest dose, 180 mg/kg, vomiting with no other signs of AChE inhibition were observed. At the next higher dose, 240 mg/kg, no adverse effects are reported.

Stanton et al. (1979) also conducted 30-day assays using only the PVC formulation.   Aside from alterations in cholinesterase activity, 30 consecutive days of exposure of young swine or gravid sows to doses as high as 25 mg/kg-day of the DDVP-PVC formulation produced no adverse effect on any physical or biochemical parameter measured. The authors suggest that the lack of serious adverse effects was related to the slow-release of DDVP from the PVC pellet (Stanton et al. 1979).

In an abstract, Singh et al. (1968) evaluated free DDVP (200 or 400 mg/day) or DDVP in V-13 pellet (800 mg/day; 9% DDVP, 91% inert [NOS]) in gravid sows. The DDVP, whether in free form or in the pellet, produced no adverse effects on the number of pigs born alive, number of pigs born dead, average birth weight, average number of pigs weaned at 35 days, or the average weanling weight. Minor gross signs of organophosphate poisoning (NOS) were observed only in the group receiving 400 mg/day free DDVP.

3.1.5. Subchronic or Chronic Systemic Toxic Effects

Subchronic and chronic toxicity bioassays have been conducted in several laboratory animal species (e.g., rats, mice, dogs, pigs, and monkeys), exploring the adverse effects of DDVP exposure by oral and inhalation routes of exposure. Generally, the toxic effects of DDVP exposure (regardless of route of administration) are due to the inhibition of AChE (Table 3-1). Consequently, plasma, erythrocyte, and brain cholinesterase activity are metrics of exposure and toxicity. Studies have demonstrated more sensitive neurological effects than cholinesterase inhibition; however, the toxicologic implications of these early biomarkers of exposure are uncertain. For example, the correlations between the relatively low level, chronic dichlorvos (DDVP) exposure and early electrophysiological changes (assessed by electrocorticogram, cortical evoked potentials, conduction velocity, and refractory periods of peripheral nerve) showed the electrophysiological parameters to be sensitive biomarkers of the exposure in humans (Desi et al. 1998).

In a long-term dietary study, rats fed diets containing DDVP for 2 years showed no signs of toxicity until the dietary exposures reached 2.5 mg/kg-day or more (WHO 1989). EPA (2000a) identified an unpublished dietary study in dogs (MRID No. 41593101 as summarized by U.S. EPA 1994) as the basis for establishing a risk level for chronic oral exposure. Groups of beagle dogs received DDVP orally in capsules at dose levels of 0, 0.1, 1.0, and 3.0 mg/kg/day for 52 weeks. The 0.1 mg/kg/day dose was lowered to 0.05 mg/kg/day on day 22 due to the inhibition of plasma ChE noted after 12 days (the magnitude of the reduction was 21.1% in males and 25.7% in females). After week 2, plasma ChE activity was only significantly reduced in males (39.1–59.2%) and females (41.0–56.7%) in the mid-dose group and in males (65.1–74.3%) and

females (61.1–74.2%) in the high-dose group at all other later time intervals. RBC AChE activity was reduced in males (23.6%) and females (50.1%) at week 6 in the low-dose group. The authors attributed this to a residual effect on RBC AChE of the earlier dose of 0.1 mg/kg/day, because much less inhibition was observed in this group after week 6. After week 6, RBC AChE activity was only significantly decreased in males (43.0–53.9%) and females (38.0–51.9%) in the mid-dose group and in males (81.2–86.9%) and females 79.2–82.5%) in the high-dose groups at all other later time intervals. Brain AChE activity was significantly reduced in males (22%) in the mid-dose group and in males (47%) and females (29%) in the high-dose group. The NOAEL and LOAEL selected by EPA (2000a) for chronic oral risk exposure are 0.05 and 0.1 mg/kg/day, respectively. These effect levels are based on plasma ChE and RBC AChE inhibition in male and female dogs as early as the first time point measure and brain AChE inhibition in male dogs.

3.1.6. Effects on Nervous System

A neurotoxicant is a chemical that disrupts the function of nerves, either by interacting with nerves directly or by interacting with supporting cells in the nervous system (Durkin and Diamond 2002). This definition of neurotoxicant distinguishes agents that act directly on the nervous system (direct neurotoxicants) from those agents that might produce neurologic effects that are secondary to other forms of toxicity (indirect neurotoxicants). As discussed in Section 3.1.2, DDVP, like all organophosphate insecticides, is a direct-acting neurotoxicant. DDVP combines with and inhibits AChE. The biochemical basis for the toxic effects of DDVP is related to the normal function of AChE. In the cholinergic system, neural impulses are transmitted between nerve cells or between nerve cells and an effector cell (such as a muscle cell) by the acetylcholine. When the acetylcholine reaches a certain level, the receptor cell is stimulated. Normally, the acetylcholine is then rapidly degraded to inactive agents (acetic acid and choline) by AChE. When AChE activity is inhibited by organophosphate agents (such as DDVP), acetylcholine persists and continues to accumulate at the synapse (the space between the nerve cells). Initially, this accumulation causes continuous stimulation of the cholinergic system, which may be followed by paralysis because of nerve cell fatigue (ATSDR 1993).

The cholinergic effects of DDVP intoxication are well documented in studies involving humans, wildlife, and experimental mammals (Gillett et al. 1972a,b; IARC 1979, 1991; WHO 1989). DDVP also inhibits other cholinesterases and many other esterases outside of the nervous system and induces clinical signs of intoxication that are dependent upon the dose and duration of exposure (Table 3-1). In addition, some studies of lifetime exposure of rats to DDVP suggest that oral exposures to doses ≥0.97 mg/kg-day result in behavioral changes (Schultz et al. 1995, Institäoris et al. 1997).

RBC AChE activity follows a circadian oscillation in both mice and humans (Jian and Zhiying 1990). Furthermore, mortality in mice associated with exposure to DDVP is inversely related to the oscillation in AChE activity. These investigators report that DDVP interferes with the normal circadian rhythm of RBC AChE in mice and humans, although this interference is secondary to pronounced AChE inhibition.

The effect of DDVP on AChE activity in humans has been assayed by Gledhill (1997). In this study, DDVP was administered to 6 male volunteers as a single dose of 70 mg DDVP in a corn oil solution in a gelatin capsule. The body weights of 6 individuals ranged from 67 kg to 80 kg (Gledhill 1997) and thus the individual dose rates ranged from 0.70 to 1.04 mg/kg bw. No effect on AChE activity was observed and there were no signs or symptoms of cholinergic overstimulation.

Normal ChE activities can be highly variable among individuals. Consequently, interpreting differences between cholinesterase levels in exposed groups and control groups is more difficult than interpreting differences between individual ChE levels before and after exposure (ATSDR 1993). All of the human and animal studies on PVC-DDVP formulations report AChE levels using the method involving treated groups and control groups. For all of the human studies on DDVP (Cervoni et al. 1969; Pena-Chavarria et al. 1969; Hine and Slomka 1970; Slomka and Hine 1981), the interpretation is further complicated because ChE levels are reported as ranges of inhibition, rather than mean values with standard errors.

As discussed in the general literature and illustrated in the human studies on DDVP, inhibition of cholinesterase in plasma and blood is not necessarily associated with clinically significant adverse effects (Gage 1967; Wills 1972). ATSDR (1997) noted that the nervous system can accept a certain amount of acetylcholinesterase inhibition without overt toxic effects. In humans and animals, toxic signs are generally not seen until at least 20% of this enzyme (RBC AChE used as a marker) has been inhibited (ATSDR 1997). In a rat study, brain AChE after a 2-year inhalation exposure to DDVP was inhibited more than 90% compared to control animals (Blair et al. 1976), yet signs of cholinergic overstimulation were not observed. ATSDR (1997) suggests that the best predictor of toxicity is not necessarily the actual percentage inhibition of AChE, but rather how rapidly this inhibition has occurred. Rapid inhibition does not afford the nervous system time to adapt to AChE inhibition. This adaptation appears to involve desensitization and down regulation of muscarinic receptors (ATSDR 1997).

A significant characteristic of some organophosphate insecticides is that the reversibility of enzyme inhibition is slow (Murphy 1980). Relatively little information is available on the reversibility of inhibition due to DDVP. There is one case report indicating substantial inhibition of ChE, 36% of normal, in an individual exposed to DDVP 3 days before the assay of ChE activity (Bisby and Simpson 1975), and other data suggest that cholinesterase activity levels do not return to normal for several months (ATSDR 1997).

Exposure to some organophosphorus compounds cause delayed neuropathy in humans (also known as organophosphate-induced delayed neurotoxicity or OPIDN). Clinical manifestations include motor dysfunction, tingling in the extremities, and in some cases paralysis. These effects usually appear 7–14 days after exposure, when signs of cholinergic toxicity have resolved, and can persist for weeks or years (ATSDR 1997). The data concerning the potential for DDVP-induced OPIDN are inconsistent and controversial. Several studies that demonstrate that DDVP does not induce delayed neuropathy (WHO 1989), including a recent study in adult hens

(Abdelsalam 1999). On the other hand, very high doses of DDVP (doses in excess of the LD_{50}) produced clinical neuropathy when administered to hens (Johnson 1978, 1981). These data are consistent with human cases of poisoning where recovery was followed by delayed neurotoxicity (see Section 3.1.4) (WHO 1989). Subcutaneous doses of DDVP (single dose of 200 mg/kg or 6 mg/kg-day for 8 weeks) in rats led to motor deficit or biochemical and behavioral deficits (Sarin and Gill 2000, 1998, respectively). The potential for OPIDN in humans resulting from exposure to DDVP in PVC resin strips is unknown.

3.1.7. Effects on Immune System

Immunotoxicants are chemical agents that disrupt the function of the immune system. Two general types of effects, suppression and enhancement, may be seen and both of these are generally regarded as adverse. Agents that impair immune responses (*immune suppression*) enhance susceptibility to infectious diseases or cancer. Enhancement or *hyperreactivity* can give rise to *allergy* or hypersensitivity, in which the immune system of genetically predisposed individuals inappropriately responds to chemical or biological agents (e.g., plant pollen, cat dander, flour gluten) that pose no threat to other individuals or *autoimmunity*, in which the immune system produces antibodies to self components leading to destruction of the organ or tissue involved (Durkin and Diamond 2002).

Although the literature contains some evidence that organophosphate insecticides can impair immunological markers (Colosio et al. 1999), no human data are available to describe a dose-response relationship for the immunotoxic potential of DDVP. Animal studies suggest that exposure to DDVP may be associated with immunosuppression. Treating rabbits with oral doses of 0.31–2.5 mg/kg DDVP (2.5–20% of the LD_{50}) 5 days per week for 6 weeks resulted in inhibition of both humoral and cell-mediated immune response to *S. typhimurium* (Desi et al. 1978, 1980). Immunosuppression (suppressed IgM response at 48 hours) was also observed in mice treated with a single oral dose of 120 mg/kg DDVP (Casale et al. 1983). A decrease in relative spleen weight was also noted in this study; however, severe signs of DDVP neurotoxicity were noted and the authors stated that the immunosuppression observed in this study may have been related to toxic chemical stress. In addition, *in vitro* studies on cells from embryonic renal tissue of carp demonstrated a dose-related decrease in lymphocyte proliferation and myeloid cell respiratory burst activities, both of which indicate immunosuppression; however, no effects on antibody production were noted in an *in vivo* study of carp (Dunier et al. 1991). Bryant (1985) has associated the precipitation of preexisting asthma to small doses (NOS) of DDVP.

Aside from the few positive reports above, there is very little direct information on which to assess the immunotoxic potential of DDVP in humans. The extrapolation of the observed alterations in the immune system response of experimental animals to humans is uncertain, since the functional relevance of these deficits in humans is unknown. The immune system has a functional reserve and modifications in the immune response do not always correlate with a measurable health effect (Vial et al. 1996; Voccia et al. 1999).

The systemic toxicity of DDVP has been adequately examined in numerous acute, subchronic, and chronic bioassays. Although many of these studies did not focus on the immune system, changes in the immune system (which could potentially be manifest as increased susceptibility to infection among DDVP-exposed animals compared to controls) were not observed in any of the available long-term animal studies. In a three-generation study of Wistar rats, neurologic endpoints were found to be more sensitive markers of exposure than immunologic endpoints in all three generations (Institäoris et al. 1997).

3.1.8. Effects on Endocrine System

In terms of functional effects that have important public health implications, some of the effects on endocrine function would be expressed as diminished or abnormal reproductive performance. This issue is addressed specifically in the following section (Section 3.1.9). As discussed in Durkin and Diamond (2002), mechanistic assays are generally used to assess the potential for direct action on the endocrine system. DDVP has not been tested for activity as an agonist or antagonist of the major hormone systems (e.g., estrogen, androgen, thyroid hormone), nor have the levels of these circulating hormones been adequately characterized following DDVP exposures. Alterations in the diurnal rhythm of the pituitary/adrenal axis were observed in rats exposed to 2 ppm (approximately 0.3 mg/kg) DDVP in drinking water. Although effects on plasma ChE activity were not noted, levels of plasma adrenocorticotrophic hormones and adrenal cholesterol ester were altered (Civen et al. 1980). In the absence of mechanistic studies of the endocrine system, any judgments concerning the potential effect of DDVP on endocrine function must be based largely on inferences from standard toxicity studies, none of which provide evidence for an endocrine effect.

3.1.9. Reproductive and Teratogenic Effects

No data are available in humans concerning the potential for DDVP-induced reproductive or developmental toxicity. As a small, lipid-soluble molecule, DDVP would be expected to cross the placental barrier and be excreted into breast milk (Desi et al. 1998). According to some studies, exposure to DDVP caused reproductive and teratogenic effects in laboratory animals; on the other hand, there are several breeding studies in which no adverse reproductive or teratogenic effects were observed in rabbits or swine after exposure to DDVP (ATSDR 1997). In a study in which female rats were given intraperitoneal injections of 15 mg/kg DDVP on day 11 of gestation, herniation of the umbilical cord was observed in 3 of 41 offspring from the treated group (Kimbrough and Gaines 1969). The effect was not observed in offspring from the control group (0/65) but the effect is not statistically significant using the Fisher Exact test (p=0.074) – i.e., the conventional criterion for statistical significance is a p-value of ≤ 0.05. In a three-generation study of Wistar rats, oral gavage doses of approximately 1, 1.3, or 1.9 mg/kg-day 5 days/week for 28 weeks found no consistent toxicity (systemic, reproductive, or immunologic) across generations (e.g., birth body weight was statistically decreased in generation 2 and increased in generation 3) (Institäoris et al. 1995, 1997).

When rabbits were treated with 6 mg/kg DDVP during the last 10 days of gestation and the brain tissue of the offspring was examined by electron microscopy, there was an incidence of

immaturity or delay in brain development that was not apparent in the offspring of the untreated rabbits (Dambska et al. 1979). The method of dosing the animals is not specified in this study. Groups of New Zealand White rabbits (16/dose) received DDVP (97% purity in distilled water) orally at dose levels of 0, 0.1, 2.5, or 7.0 mg/kg/day on gestation days 7 through 19 (U.S. EPA 2000a, p. 19). The NOAEL for maternal toxicity was 0.1 mg/kg/day and the LOAEL was 2.5 mg/kg/day, based on decreases in maternal body weight gain during gestation days 7–19. The U.S. EPA (2000a) considered the decrease in weight gain to be biologically significant even though the effect was not statistically significant. A dose-related increase in maternal mortality also was noted at 2.5 and 7 mg/kg/day. Cholinergic signs were observed at 7 mg/kg/day. No adverse developmental effects were noted in the fetuses. Cholinesterase activity was not determined.

An early study by Schwetz et al. (1979) in New Zealand White rabbits and CF-1 mice using the MTD dose (based on signs of cholinesterase inhibition) for both oral (gavage of 5 mg/kg-day DDVP in corn oil on gestation days 5–18 and 60 mg/kg-day DDVP in corn oil on gestation days 5–16 for rabbits and mice, respectively) and inhalation (whole body exposure to atmospheres containing 4 ì g/dL (0.4 mg/L or 400 mg/m^3) DDVP for 7 hours/day on gestation days 5–18 or 5–16 for rabbits and mice, respectively) routes of exposure found no teratogenic effects that could be attributed to DDVP. These studies suggest that DDVP is not a selective developmental toxin, since adverse developmental effects only occur at doses that are maternally toxic.

At toxic doses (i.e., where signs of organophosphorus poisoning are evident), DDVP may produce reversible adverse effects on spermatogenesis (WHO 1989). Adverse testicular effects were observed in mice after chronic exposure to average daily doses of 0, 58, or 94.8 mg/kg/day DDVP in drinking water (MRID 41041801 as cited by U.S. EPA 1994). There was a dose-related decrease in the absolute and relative weight of the testes, and testicular atrophy was increased at 94.8 mg/kg/day. In addition, sperm abnormalities were seen in C57BL/C3H mice injected intraperitoneally with 10 mg/kg/day for 5 days (Wyrobek and Bruce 1975). About 6% of the sperm from DDVP-treated animals was abnormal compared to 1.8% of sperm from untreated animals. In a reproductive toxicity study involving male CF-1 mice, groups of 16 mice were exposed to atmospheres containing 0, 30, or 55 mg/m³ (0, 3.3, or 6.1 ppm, respectively) for 16 hours or to 0, 2.1, or 5.8 mg/m³ 23 hours/day for 4 weeks (Dean and Thorpe 1972). No differences between control and treated mice were observed in the number of early fetal deaths, late fetal deaths, or live fetuses found in the pregnant females. The percentage of pregnancies for females mated to males exposed to DDVP was also similar to the controls (73–88%, mean 80.9%). Under these exposure conditions, DDVP does not appear to affect the fertility of male CF-1 mice. No gross or histological evidence of treatment-related damage to reproductive tissues (prostate, testes, epididymis, ovaries, or uterus) was seen in F344 rats (4 or 8 mg/kg/day) or B6C3F1 mice (10, 20, or 40 mg/kg/day) orally exposed to DDVP by gavage for 2 years (NTP 1989).

3.1.10. Carcinogenicity and Mutagenicity

Adequate data regarding the carcinogenic potential of DDVP in humans by any route of exposure are not available. Studies of human populations exposed to DDVP (including workplace and residential exposures) are constrained by the lack of adequate exposure data and other limiting issues. As reported in a series of case studies, some evidence suggests an association between childhood cancer and exposures to DDVP in resin strips during childhood or during gestation (Reeves et al. 1981, Davis et al. 1992, 1993, Liess and Savitz 1995). These studies have been reviewed by U.S. EPA (2000a) which concluded:

> "*[r]eviews of these studies have identified biases and confounders that could explain the observed associations. The Agency concludes that the biases are a more likely explanation for the findings of increased cancer than exposure to resin strips. Additional studies that correct for the control of potential biases and problems of exposure determination are needed before an association between Dichlorvos and childhood cancer can be established*" (U.S. EPA, 2000a, p. 26).

The carcinogenic potential of DDVP has been evaluated in several animal species (mice, rats, dogs, and swine) via the oral route and in rats via the inhalation route. The weight of evidence suggests that the cancer bioassays do not offer sufficient evidence to treat DDVP as a potential human carcinogen (U.S. EPA 2000a,b). DDVP produced positive results in mammalian bioassays for carcinogenicity by the oral, but not the inhalation route of exposure. A cancer bioassay was conducted in which male and female mice were given gavage doses of DDVP (NCI 1977). The doses levels were 10 and 20 mg/kg for males and 20 and 40 mg/kg for females. There was a significant dose-related increase in squamous-cell papillomas of the forestomach in both sexes. In females at the high-dose level, the incidence of squamous-cell carcinomas was significantly greater than in the control group ($p=0.004$ using the Fisher Exact test). In the same study, male rats were given 4 mg/kg/day DDVP by gavage and female rats were given 8 mg/kg/day. A significant ($p<0.001$) dose-related increase in the incidence of acinar-cell adenomas of the pancreas was observed in the males. The increased incidence of fibroadenomas and adenomas of the mammary gland was significant ($p=0.028$) in the females. The increased incidence of the pancreatic acinar cell carcinomas in male rats and squamous cell tumors in male mice reported by NCI (1977) has been discounted by WHO (1989) and Mennear (1994, 1998). The relevance of the sex-specific increase in mononuclear cell carcinoma (MCL) reported by NCI (1977) has also been questioned (Manley et al. 1997, Mennear 1998, U.S. EPA 2000b). The issues of concern regarding the increased incidence of MCL in male rats are not dose-related increases in mortality or disease severity (Mennear 1998), incidence rates among DDVP-treated rats statistically increased as compared to matched controls but within historical control incidence, and similarity in histopathology between the MCL tumors and spontaneous tumors in control animals (Manley et al. 1997). U.S. EPA (2000b) found compelling evidence to disregard the MCL finding in Fisher rats, concluding that "*the high background and variability in the incidence of this tumor, as well as its species and strain specificity, make it an invalid response for human risk assessment*". Two other bioassays conducted on the carcinogenicity of DDVP

after oral exposure are reviewed by IARC (1991). Neither study indicated significant evidence of carcinogenicity (IARC 1991).

DDVP has been tested extensively for mutagenicity, and the results of the tests are available in several reviews (IARC 1979, 1991, Ramel et al. 1980, Mennear 1998, U.S. EPA 2000a,b). Mutagenic effects as well as covalent binding to RNA and DNA have been demonstrated in bacterial systems. Generally, mutagenicity is decreased by the presence of liver microsomal preparations; however, chromosome abnormalities in peripheral lymphocytes have been reported in pesticide workers who use DDVP (no quantitative exposure data are available and this appears to be from workers using a spray formulation of DDVP) (Desi et al. 1998). EPA (2000b) concluded that "*the results from whole animal bioassays supercede the results in vitro tests... [C]ompounds that are positive in mutation tests but do not cause cancer in whole animals should be regulated as noncarcinogens*".

A more detailed review of the cancer and mutagenicity literature database on DDVP is beyond the scope of this risk assessment. Owing to the extraordinary level of effort and Special Agency Reviews of the issue (U.S. EPA 2000a,b), this risk assessment will defer to the EPA's latest position (U.S. EPA 2000a) concerning the carcinogenic and mutagenic potential of DDVP. In that assessment (U.S. EPA 2000a), which included an open meeting to discuss the issues (U.S. EPA 2000b), it was decided that "[*t*]*he carcinogenicity potential of Dichlorvos has been classified as 'suggestive' under the 1999 Draft Agency Cancer Guidelines and no quantitative assessment of cancer risk is required*". Thus, this risk assessment for DDVP does not include a quantitative assessment of cancer risk.

3.1.11. Irritation and Sensitization (Effects on the Skin and Eyes)

The available human data, supported by studies on experimental animals, suggest that exposure to DDVP may cause skin irritation or allergic reactions. Human data regarding the dermal effects of DDVP are relatively sparse. In a case report, relatively severe contact dermatitis developed in an adult male after a 1% solution of DDVP leaked onto his skin (Bisby and Simpson 1975). This effect was accompanied by signs of cholinergic toxicity, including fatigue, dizziness, and labored respiration. Cases of dermatitis and skin sensitization due to DDVP have been described in workers handling and spraying different types of pesticides and cross-sensitization with certain pesticides has been seen (WHO 1989).

The data from animal testing supports the results of human case reports. In New Zealand white rabbits, the application of an aqueous solution of 5–20% DDVP to the skin caused relatively severe irritation (Arimatsu et al. 1977). In a skin sensitization assay, 1% DDVP in olive oil induced no visible effects in male albino guinea pigs (Kodama 1968). In a guinea pig assay for allergenicity, 35% of the tested guinea pigs had a positive response to a 0.5% solution of DDVP (Fujita 1985). In a sensitization assay, Ueda et al. (1994) reported that 1% DDVP was a threshold irritation concentration in guinea pigs and that cross-sensitization occurred between DDVP and triforine. WHO (1989) reported that in Hartley guinea pigs the primary irritant threshold limit value for DDVP was $\geq 2\%$.

3.1.12. Systemic Toxic Effects from Dermal Exposure

Most of the systemic effects observed after dermal exposure of laboratory animals (including monkeys, rats, and chickens) to DDVP were the result of the neurotoxicity of this chemical. In its risk assessment for DDVP, U.S. EPA (2000a) selected studies for short-term and intermediate-term risk assessment that reflect the systemic toxicity resulting from dermal exposures to DDVP. In both of these studies, the toxicity of DDVP is secondary to inhibition of cholinesterase activity. Data concerning the dermal absorption kinetics of DDVP are discussed in Section 3.1.3.2.

A number of fatalities have been reported from dermal exposures to concentrated formulations of DDVP (spilling or splashing onto skin) (WHO 1989). The data suggests that, in those cases where the spilled solution was immediately washed off, the victims developed symptoms of organophosphorus poisoning but they recovered after treatment (WHO 1989). Such exposures are not relevant to this risk assessment, as the encapsulation of DDVP in PVC used in Vaportape II precludes rapid exposure to high doses of DDVP.

3.1.13. Inhalation Exposure

Exposure of pesticide manufacturing plant workers to concentrations in the air of up to 0.5 mg/m³ were without clinical effects, and no, or only insignificant, inhibition of blood ChE activity was noted (WHO 1989). When DDVP is used properly, air levels of 0.01–0.03 ppm are achieved (ATSDR 1997). This level kills most insects within 1 hour; whereas, in human volunteers, exposure at about 20 times this level (0.23 ppm) for 2 hours a day for 4 days had no harmful effects (ATSDR 1997). Consistent with the human exposure data, harmful effects have not been seen in laboratory animals exposed to air levels of dichlorvos below 0.5 ppm (about 4.5 mg/m³) (ATSDR 1997), and exposure of laboratory animals to DDVP air concentrations between 0.2–1 mg/m³ do not affect ChE activity significantly (WHO 1989). In a 2-year study in rats, breathing air every day containing low-to-moderately high levels (0.006–0.6 ppm or about 0.05 to 5 mg/m³) of DDVP had no effect on survival or general health (ATSDR 1997). Generally, the systemic effects observed after inhalation exposure of laboratory animals to higher levels of DDVP were the result of the neurotoxicity (cholinesterase inhibition) (U.S. EPA 2000a). Chronic inhalation exposure of laboratory animals to DDVP produced no compound-related pulmonary toxicity (U.S. EPA 2000a).

EPA (1994) selected the chronic inhalation study in rats (Blair et al. 1976) as the basis for establishing an RfC for DDVP. Groups of 50/sex/group Carworth E Farm (CFE) rats were exposed (whole body exposures) for 23 hours/day, 7 days/week to DDVP vapor (>97% purity) at atmospheric concentrations of 0, 0.05, 0.5, and 5 mg/m³ for 2 years. The rats were observed for clinical signs of toxicity, hematology, and clinical chemistry. Plasma, RBC, and brain cholinesterase activity were determined at study termination, but not prior to the study. No clinical signs of toxicity were observed, and no organ weight or organ to body weight changes or hematological changes were associated with DDVP exposure. Body weights were decreased as compared to control rats in high-dose male (up to 20% vs. control) and female rats (up to 14% vs. control) for large portions of the study. Dose-dependent reductions in plasma, RBC, and

brain cholinesterase activity were observed. This study establishes a NOAEL of 0.05 mg/m³ and a LOAEL of 0.5 mg/m³ based on reductions in brain cholinesterase activity (U.S. EPA 2000a).

3.1.14. Inerts and Adjuvants
As discussed in Section 2.2, the DDVP used in gypsy moth control programs is contained in a multi-layered polyvinyl chloride (PVC) strip. The manufacturer (Hercon 2004) indicates that the product contains 10% DDVP, 0.75 % related compounds (Section 3.1.15), and 89.25% inert ingredients. The only toxicity data available on this strip itself (i.e., without DDVP) is an acute oral toxicity study in rats (Braun and Killeen 1975). This study used a DDVP-free strip ground to a "grayish-green powder". The strip was tested at the limit dose of 5,000 mg/kg bw by gavage with a 14-day post-dosing observation period in 5 male and 5 female rats. No adverse effects were noted in any of the rats based on mortality, gross observations, body weight gain, and gross necropsy. While this single study has its limitations, it suggests that the PVC strip alone (i.e., without DDVP) is unlikely to produce acute adverse effects. Given the limited nature of the exposure scenarios assessed herein, these data may be sufficient information for the likely exposure scenario (i.e., a child putting a strip in his/her mouth). Section 3.1.17 focuses on the toxicity studies concerning DDVP embedded in the PVC strips.

3.1.15. Impurities and Metabolites
The product label Hercon (2004) specifies that, in addition to DDVP (10%), each strip contains 0.75% compounds that are related to DDVP. Further details are not provided on the label; nonetheless, the impurities in commercial DDVP have been characterized (Gillett et al. 1972a; IARC 1991). The impurities include: Dipterex (O,O-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate); O,O-dimethyl 2-chlorovinyl phosphate; O,O-dimethyl methylphosphonate; O,O,O-trimethyl phosphate; and trichloroacetaldehyde. These impurities are known to be or are likely to be toxic (Gillett et al. 1972a, WHO 1989). These impurities are encompassed in the risk assessment because the effect levels are based on studies that used commercial grade DDVP. Consequently, the results of these studies are directly applicable to the risk assessment for human health.

3.1.16. Toxicologic Interactions
The major toxicologic interaction of concern is concurrent exposure to other cholinesterase inhibitors (e.g., organophosphate or carbamate insecticides) or cholinomimetic agents (e.g., agents such as pilocarpine or carbachol that mimic the action of acetylcholine). In either case, simultaneous exposure would likely enhance the cholinergic toxicity produced by DDVP. Potentiation studies using DDVP in combination with 22 other organophosphate pesticides, however, found little or no potentiation (WHO 1989). Chemicals that react with the serine residue at the active site of the "A"-type esterases (e.g., diisopropylfluorophosphate [DEP]) could also increase the toxicity of DDVP by interfering with its metabolism (ATSDR 1997).

In addition, experimental data suggest that repeated exposures of rats to DDVP (5 mg/kg/day by intraperitoneal injection for 30 consecutive days) depletes brain glutathione levels (Julka et al. 1992). Reduced glutathione levels may decrease the rate of detoxification of DDVP by the

glutathione-dependent metabolic pathways. The toxicologic significance of depleted brain glutathione on DDVP metabolism is not known. In contrast with the potentiation of DDVP toxicity observed when rats are pretreated with diethylmaleate (Fukami 1980), Costa and Murphy (1984) reported that pretreatment with 600 mg/kg acetaminophen (which is also detoxified by and thus reduces glutathione levels) did not have any effect on the toxicity of DDVP. Although no data are available, these experiments suggest that repeat exposure to DDVP (resulting in a depletion of glutathione levels) may increase an organism's susceptibility to toxicity by another chemical if that chemical is also detoxified by glutathione-dependent pathways.

3.1.17. Studies on PVC Formulations of DDVP

In the EPA risk assessment for DDVP (U.S. EPA 2000a), EPA noted that DDVP resin strips (such as the Vaportape II strip used in USDA programs) "*account for a very small proportion of total incidences* [e.g., reports of poisonings]*, about 33 cases per year (1% of total incidences). Incidence reports involving exposure to resin strips usually do not involve any significant acute symptoms that would require medical treatment*". In a review of DDVP-impregnated PVC strips (Gillett 1972a,b concluded that "*even when chewed or applied directly to the skin for short intervals, the strips do not release excessive or hazardous amounts of DDVP*".

When DDVP was administered orally to human volunteers (single or repeated doses of a slow-release PVC formulation), significant inhibition of RBC ChE activity was found only at 4 mg/kg body weight or more (Hine and Slomka 1970; Slomka and Hine 1981). Single oral doses (1–32 mg/kg) of DDVP in a slow-release PVC formulation was administered to 107 male volunteers. Measurable reductions in erythrocyte ChE activity was observed at dose levels above 4 mg/kg, with a maximum reduction of 46% at 32 mg/kg. Plasma ChE activity was affected at lower doses, with 50% reduction at 1 mg/kg and about 80% reduction at 6 mg/kg or more. Repeated oral doses of 1–16 mg/kg bw per day were given to 38 male volunteers for up to 3 weeks. Plasma ChE activity was depressed at all dose levels, and RBC AChE activity depression was dose-related and statistically significant at doses of 2 mg/kg or more. Blood cell count, urine, liver function, prothrombin time, and blood urea nitrogen were all normal (Hine and Slomka 1968, 1970, Slomka and Hine 1981, WHO 1989). Among these individuals, the clinical signs of DDVP exposure were minimal (nausea, diarrhea, lassitude, restlessness, and light-headedness).

Data from 32 rhesus monkeys receiving orally administered DDVP in PVC resin (as an anthelminthic) at 0, 5, 10, 20, 40, or 80 mg/kg once daily or 0, 8, or 20 mg/kg twice daily for 10 to 21 days support the human data (Hass et al. 1971). None of the monkeys died or exhibited debilitating symptoms of organophosphorus poisoning, although some cholinergic effects were noted (a loss of appetite and emesis [LOAEL = 20 mg/kg]; diarrhea and salivation [LOAEL = 80 mg/kg]). A semi-quantitative assay for cholinesterase activity demonstrated inhibition. Studies in swine treated with DDVP-impregnated pellets (veterinary use as anthelminthic) suggest that DDVP is absorbed from the pellets after oral exposure (Jacobs 1968, Potter et al. 1973). Neither study was reported in sufficient detail to develop dose-response relationships.

Two reproduction studies investigated exposure to PVC-DDVP formulations. In one of the studies, swine were exposed to 5 or 25 mg/kg/day DDVP during the last 30 days of gestation (Stanton et al. 1979). Sows and fetuses were monitored for changes in ChE. Both plasma ChE and RBC AChE were inhibited in sows, and brain AChE was increased in fetuses. In a separate experiment conducted by these investigators, there were no significant effects on reproductive capacity in sows treated with 25 mg/kg/day DDVP during the last 30 days of gestation. In an abstract concerning DDVP encapsulated in PVC, Vogin (1971) reported that no adverse effects on reproduction or developmental parameters were observed in dams exposed to DDVP concentrations that did not cause maternal toxicity (up to 12 mg/kg). Maternal toxicity was evident in dams treated with 34 mg/kg. This abstract also employed exposures to PVC resin and dioctylphthalate to assess the potential developmental toxicity of inerts. No teratogenic effect was reported for any exposure regimen.

When DDVP pesticide strips were used in hospital wards, exposure of hospitalized adults and children, as well as healthy pregnant women and newborn babies, did not produce any significant effects on plasma ChE or RBC AChE activity. Exposures were estimated TWA concentrations of 0.05, 0.152, and 0.159 mg/m³ based on 18 hours/day (Vigliani 1971). Only those subjects exposed 24 hours/day to concentrations above 0.1 mg/m³ or patients with liver insufficiency showed a moderate decrease in plasma ChE activity (Cavagna et al. 1969). Cavagna et al. (1969) also calculated DDVP inhalation exposure doses (based on inhalation volumes of 10 m³/day for adults and 1.4 m³/day for children and continuous exposures) that would be required to produce a significant reduction in plasma ChE activity (25–54% reduction in activity) for healthy adults and children (approximately 0.03 mg/kg-day) and adults and children with liver insufficiency (approximately 0.006 mg/kg-day). Note that these exposure doses are not anticipated to produce signs or symptoms of cholinesterase inhibition (Cavagna et al. 1969). No significant effects on plasma ChE or RBC AChE activity were observed in people exposed to the recommended rate of one strip per 30 m³ in their homes over a period of 6 months, even when the strips were replaced at shorter intervals than that normally recommended (Zavon and Kindel 1966). The maximum average concentration in the air of the homes was approximately 0.1 mg/m³ (WHO 1989). In factory workers exposed to an average of 0.7 mg/m³ for 8 months, significant inhibition of plasma ChE and RBC AChE activity was found (WHO 1989).

In a study evaluating the effects of 30 minutes of dermal exposure to a DDVP pest strip on AChE activity, no dermal effects were noted in 21 individuals (Zavon and Kindel 1966). Zavon and Kindel (1966) also reported no inhibition of plasma or erythrocyte cholinesterase from the 30 minute dermal exposure as well as 5 consecutive days of 30 minutes of continuous dermal exposure to DDVP resin strips. EPA (1981) provides a summary or exposure incidents involving DDVP in the general public. The reports involving DDVP-impregnated resin strips involved dermal contact which largely resulted in DDVP-induced allergic reactions or contact dermatitis (this is consistent with the effects of DDVP reported in dermal contact bioassays as described in Section 3.1.12). Flea collar dermatitis (primary contact dermatitis) has been reported in dogs and cats wearing DDVP-impregnated PVC flea collars (Muller 1970), and four people who handled dogs wearing flea collars containing 9–10% DDVP developed contact dermatitis (patch tests

using 0.25–1% DDVP in these individuals were positive). The data suggest that a very small proportion of the general population is susceptible to dermal irritation by DDVP (WHO 1989).

3.2.   EXPOSURE ASSESSMENT
3.2.1. Overview.
Under normal conditions, exposure to both workers and members of the general public should be negligible. Workers will handle strips only during the assembly of milk carton traps. If workers wear gloves and assemble the traps outdoors or in very well ventilated rooms, both inhalation and dermal exposures should be negligible. Inhalation exposure to DDVP during transport of the traps should also be negligible if the traps are not transported inside of the passenger compartments of vehicles. Worker exposures will also be limited in most programs because foil wrapping in which the strip is distributed will not be removed until after the trap is transported to the field.   Milk carton traps will generally be placed about four feet above the ground and exposure of members of the general public to DDVP contained in the milk carton traps should also be negligible except in the case of intensional tampering.

Notwithstanding the above assertions, exposure assessments are developed for workers who do not use gloves in the assembly of the milk carton traps and who assemble the traps indoors, remove the protective foil strip during assembly, and transport the traps in the passenger compartments of vehicles. All of these exposure scenarios should be considered atypical and some are extreme. The intent is to illustrate the consequences of mishandling or imprudent handling. During assembly, the central estimate of dermal exposures in workers not wearing gloves leads to an absorbed dose of about 0.0008 mg/kg with a range of about 0.0003 mg/kg to 0.004 mg/kg. Inhalation exposures in workers may be highly variable depending on the ventilation rates in an enclosed space and the number of traps that are handled. Based on the handling and transport of 75 traps, inhalation exposures could reach up to about 0.6 mg/m^3 in an enclosed and unventilated room and up to about 1.8 mg/m^3 in the passenger compartment of a vehicle. These exposure assessments are based on several site and situation specific assumptions which are intended to reflect plausible upper bounds of exposures.

Exposure assessments are also developed for children who might come in contact with an accidentally discarded or misplaced strip. Estimated dermal doses are much higher than those for workers: a central estimate of about 0.02 mg/kg with a range of 0.003 mg/kg to 0.1 mg/kg. Oral exposures from a small child sucking on the pest strip are about a factor of 10 higher than dermal exposures: a central value of about 0.2 mg/kg with a range of 0.04 mg/kg to 0.6 mg/kg.

Under normal circumstances, the use of DDVP in PVC strips is not likely to result in contamination of water or other materials that might be consumed by members of the general public. Nonetheless, an exposure assessment is developed for the accidental contamination of a small pond by a pest strip. In this scenario, dose estimates range from about 0.000003 mg/kg to 0.00007 mg/kg with a central estimate of about 0.00001 mg/kg.

3.2.2. Workers
3.2.2.1. General Considerations –   The EPA (2000a) concluded that human exposures would be negligible from DDVP-impregnated strips in insect traps (such as those used in USDA programs). Consequently, the EPA (2000a) did not quantitatively assess the exposure or

potential risks posed by the use of PVC formulations of DDVP for any route of exposure. While this may be a reasonable approach, the current risk assessment develops quantitative exposure assessments for both workers and the general public that could occur in cases of poor handling practices.

The milk carton traps can be assembled in two stages. The most time consuming stage is the carton assembly, in which two pre-cut perforated pieces of heavy waxed paper, similar to those used in milk cartons, are configured. In the second stage, the DDVP strip and disparlure wick are attached to the twist tie, and the twist tie is placed in the trap. The second stage should proceed much more rapidly than the first. During assembly, two routes of exposure may be significant, inhalation and dermal. As discussed in the program description (Section 2.2), however, both routes of exposure will be negligible if proper handling procedures are followed (that is, if the strips are installed outdoors or in a well ventilated area, if foil wrapping in which the strip is distributed is removed until after the trap is transported, and dermal contact with the strip is avoided).

3.2.2.2. Inhalation Exposures – During normal use and assembly, either outdoors or in well ventilated areas, inhalation exposures to DDVP should be negligible. The material safety data sheet for VaporTape II (Hercon 1993) calls for local exhaust and respirators under conditions of continuous handling. Estimates of concentrations of DDVP in air from release of DDVP by VaporTape strips under different conditions of ventilation can be based on estimates of release rates (Hercon 1994) and a more general air model for DDVP pest strips proposed by Gillett et al. (1972a).

Hercon (1994) conducted a study on the release of DDVP from Vaportape II strips. In this study, two samples (referred to as **A** and **B**) were weighed and assayed for DDVP at various intervals for up to 12 weeks after placement outdoors. The results, expressed as the proportion of DDVP remaining in the strip at various intervals, are detailed in Worksheet A01. As also detailed in Worksheet A01, the release data fit a first order model extremely well with an adjusted squared correlation coefficient of 0.97 and a p-value of 2×10^{-23}. The estimated first-order release coefficient is 0.04 day^{-1} with very narrow confidence intervals – i.e., 0.037 to 0.043 day^{-1}.

Gillett et al. (1972a) proposed the following model for estimating concentrations of DDVP in air from the release of DDVP from pest strips:

$$
C_t = \frac{8}{\pi^2}\ \frac{M_0}{Va(1+\gamma)}\ \frac{\exp(-\lambda t)\ -\ \exp(\dfrac{-(kRH+\dfrac{At}{Va})}{1+\gamma}\ t)}{\dfrac{(kRH+\dfrac{At}{Va})}{\lambda\ (1+\gamma)\ -1}}
\qquad\text{(Eq. 3-1)}
$$

The terms in the above equation are defined as follows:

t time after start of release

C_t concentration of DDVP in air at time, t (days)

M_0 mass of DDVP in strip or strips at time zero (mg)

Va volume of room or other space (m^3)

$\tilde{a}$ apparent adsorption coefficient of DDVP on to surfaces

$\exp(x)$ the exponential function, e^x, where e is the constant 2.718 and x is any numeric expression

$\ddot{e}$ first-order release rate constant (days^{-1})

RH relative humidity (proportion)

A_t air flow rate (m^3/day)

k first-order vapor phase hydrolysis rate (days^{-1})

The parameters used in the model are summarized in Table 3-2. The fit of the Gillett et al. (1972a) model to the data from Slomka (1970) using the apparent adsorption coefficient ($\tilde{a}$) of 37.5 is illustrated in Figure 3-1 (which is in turn taken from Worksheet A02b). Technical details of the application of the model and optimization of the model parameter for adsorption ($\tilde{a}$) are given in Appendix 1.

For the current risk assessment, two scenarios are considered for inhalation exposures of workers to DDVP: assembly of traps with strips in a garage and driving in a vehicle containing assembled traps with the strips. Both scenarios assume that the worker has removed the protective foil from the strip during assembly of the trap. These exposure scenarios are detailed in Worksheets A03a (garage) and A03b (vehicle). It should be noted that these exposure assessments are based on a number of plausible but conservative exposure assumptions – i.e., number of traps assembled or transported, volume of the space in which the traps are assembled or transported, and the ventilation rates of these spaces. The worksheets in which these exposure assessments are given are designed so that these parameters may be varied and applied to specific uses of the DDVP strips in specific USDA programs.

A major factor in exposure will be the number of traps that are assembled. In the previous risk assessment (USDA 1995a), it was assumed that a workers would assemble up to 75 traps at a time. No more recent information has been encountered on the number of traps that might be assembled by a worker or workers and the value of 75 traps is maintained in the current risk assessment.

For exposures in a garage involving the assembly of the milk carton traps, the dimensions of the garage are assumed to be 1,500 ft^3 (10 feet · 10 feet · 15 feet) or 42.48 m^3 [1 ft^3=0.02832 m^3]. For the exposure assessment involving transport of the strips in a vehicle, the volume of the

driving cabin is assumed to be 160 ft^3 (8 feet · 5 feet · 4 feet) or 4.5 m^3. Again, these assumptions are somewhat arbitrary but are identical to the assumption used in the previous risk assessment (USDA 1995a).

The other major assumptions used in these exposure scenarios involve ventilation rates and release rates. The release rate is taken as 0.04 day^{-1} from the study by Hercon (1994) discussed above and detailed in Worksheet A01. It should be noted that the study by Hercon (1994) was conducted outdoors over a period of 12 weeks. Hercon (1994) does not specify the average temperature or range of temperatures. As discussed in Gillett et al. (1972a), the release rate of DDVP from PVC test strips will increase with increasing temperature, doubling from a temperature of 25°C to 38°C. This variability is not explicitly incorporated into the model used in this risk assessment (Eq. 3-1) and release rates higher than 0.04 day^{-1} are possible at high ambient temperatures.

Ventilation rates are likely to be highly variable. In most cases, it is likely that the milk carton traps will be assembled outdoors and will be transported in a cargo area and not in the driving cabin. In such cases, inhalation exposure would likely be negligible. For the purpose of illustrating the consequence of assembling traps in a garage or similar structure or transporting assembled traps in a vehicle, three ventilation rates (number of air turnovers per day) are used for each scenario. Rates of 0 day^{-1} (no ventilation) and 60 day^{-1} (poor ventilation) are used in both scenarios. An additional rate of 300 day^{-1} is used in the garage scenario and an additional rate of 3000 day^{-1}is used in the vehicle scenario. These rates are referred to as *"Adequate"* in Worksheets A03a and A03b. As discussed further in Section 3.4.2, this term is used because these ventilation rates lead to concentrations in air that are about 0.1 mg/m^3, the chronic NOAEL from animal studies and the TLV recommended by ACGIH (2004).

As detailed in Worksheet A03a, the garage scenario models concentrations over a 24 hour period. This duration period is selected under the assumption that traps might be stored for a day prior to use. The modeled concentrations reach up to about 0.5 mg/m^3 for no ventilation and 0.3 mg/m^3 for poor ventilation. As noted above, peak concentrations of 0.1 mg/m^3 are obtained with a ventilation rate of about 300 day^{-1}. The vehicle scenario (Worksheet A03b) covers a period of only 6 hours. It is likely that the duration of transport would typically be much less. Peak concentrations are somewhat higher – 1.8 mg/m^3 for no ventilation and about 1.5 mg/m^3 for poor ventilation. It is unclear if the no ventilation or poor ventilation assumptions are reasonable for a vehicle. As discussed by Fedoruk and Kerger (2003), concentrations of volatile organic compounds in vehicles suggest that substantial air turnover rates are likely in vehicles even when the ventilation system is turned off and the windows are closed. Quantitative estimates of air turnover rates in vehicle passenger cabins, however, have not been encountered. Nonetheless, it seems that turnover rates of 0 day^{-1} or 60 day^{-1} will lead to overestimates of concentrations of DDVP in the air of passenger compartments. Adequate ventilation for a vehicle is defined as a turnover rate of 3000 day^{-1}, the rate required to reach a concentration in air of about 0.1 mg/m^3.

3.2.2.3. Dermal Exposures – For assessing the likelihood of systemic toxic effects from dermal exposures, such as handling a pest strip during assembly, some estimate of absorbed dose is necessary. The method for making such an assessment for DDVP test strips, however, is highly uncertain.

As an individual manipulates the strip, some material will be transferred to the surface of the skin. Some of the chemical will be absorbed and some will volatilize. Assuming that the nature of the manipulation is such that a film of DDVP is maintained on the contaminated surface, Fick's first law may be used to estimate absorption (U.S. EPA/ORD 1992). Fick's first law requires an estimation of the K_p in cm per hour, the concentration of the chemical in a solution in contact with the skin, the area of the body surface that is contaminated, and the duration of exposure. There is no experimentally determined K_p for DDVP. Based on structure-activity relationships proposed by the U.S. EPA/ORD (1992), K_p for DDVP is may be estimated at about 0.00090 cm/hour with a 95% confidence interval of 0.00061 cm/hr to 0.0013. Details of these calculations are given in Appendix 2.

In this and other similar scenarios considered in this risk assessment, the DDVP is not in solution; instead, the skin is in contact with neat or undiluted DDVP. Following the recommendations of U.S. EPA/ORD (1992), the functional concentration of DDVP on the surface of the skin is assumed to be the solubility of DDVP in water, 10 mg/mL (Table 2-2) – i.e., the concentration of DDVP in pore water of the skin will be limited by the solubility of the chemical in water.

For workers wearing gloves, dermal absorption will be negligible. For workers who do not wear gloves, it is possible that the tips of the fingers and perhaps other surfaces on the hands would be contaminated. The most likely surface for contamination would be the finger tips. The precise area that might be contaminated, however, is difficult to estimate. The finger tip of each digit will be taken as 1 cm^2, except for the thumb that will be taken as 2 cm^2. Thus, the total surface area of the finger tips of both hands will be taken as 12 cm^2. This value will be used to calculate both lower and central estimates of absorbed dose. To account for the potential contamination of other parts of the hand, the upper range of exposed surface area will be taken as 24 cm^2. The duration of exposure is difficult to estimate. Most of the time spent in assembling the milk carton trap will not involve the DDVP strip. For this exposure assessment, a central estimate of 0.5 hours of total contact time with the strip is used and the range is taken as 0.25 hours to 1 hour. As detailed in Worksheet B01a, the assumptions used in this exposure scenario lead to estimates of absorbed dose of about 0.0008 mg/kg with a range of about 0.0003 mg/kg to 0.004 mg/kg.

3.2.3. General Public

3.2.3.1. General Considerations – Milk carton traps contain the strip of Vaportape II attached to a twist tie or simply placed in the bottom of the trap. The DDVP strip can be accessed easily and removed. As summarized by U.S. EPA (2000a, p. 26), incidents involving contact with DDVP resin strips have been reported but these incidents account for only a small proportion of the total

incidents involving DDVP (1% or about 33 cases per year) and the reported incidents involving DDVP strips typically to do not lead to overt signs of toxicity that require medical treatment.

In the current risk assessment, two routes of exposure are considered for the general public: dermal contact and ingestion. Milk carton traps will generally be placed about four feet above the ground (Leonard 2004) and exposure of members of the general public to DDVP contained in the milk carton traps should also be negligible except in the case of intensional tampering. Although any member of the general public could tamper with a trap, incidents such as these seem to be more plausible for children, compared with adults. While the traps may be place out of the reach of young children, the potential for exposure to the DDVP strip could occur if traps were accidentally dislodged or misplaced. In addition, using children as the exposed group is conservative because dose estimates for children, in units of mg/kg body weight, will be higher than those for adults.

3.2.3.2. Dermal Contact – The exposure assessment for dermal contact with a VaporTape II strip is detailed in Worksheet B01b. This scenario is very similar to that for dermal contact in a worker (Worksheet B01a). The major differences involve body weight, the dermal surface area that is considered, and the duration of exposure. The body weight is taken as 13.3 kg, the standard value for a 2-3 year old child (U.S. EPA/ORD 1996). In this scenario, it is assumed that a young child comes in contact with a pest strip and holds the strip against the surface of the skin for a period of time. Thus, the exposed skin surface area is taken as the dimensions of the strip – i.e., 1" x 4" inches or about 26 cm^2). The duration of exposure must be set somewhat arbitrarily. It does not seem reasonable to assume that a 2-3 year old child would be unsupervised for a prolonged period of time. Consistent with the approach taken in the 1995 risk assessments (USDA 1995a), the central estimate of exposure will be taken as 1 hour with an upper range of 4 hours. In the current risk assessment, a lower range of 15 minutes (0.25 hours) is also used and may be a more reasonable estimate of a plausible duration of exposure. Other assumptions and calculations are identical to those in the corresponding worker exposure assessment (Worksheet B01a, Section 3.2.2.3). As indicated in Worksheet B01b, this exposure assessment for a young child handling a DDVP strip leads to an estimated dose of about 0.02 mg/kg with a range of 0.003 mg/kg to 0.1 mg/kg.

3.2.3.3. Oral Exposure to DDVP Strip – As with dermal exposure, it is unlikely that children would experience any oral exposure to DDVP strips. The strips are placed within the milk carton traps and 2-3 year old children will generally be closely supervised. Thus, this exposure assessment for oral exposure, as with the above scenario for dermal exposure, should be regarded as accidental.

An assessment of oral exposure might be based on incidental sucking on a pest strip. The amount of DDVP that a child might absorb will depend on the proportion of the strip that is in the mouth, the release rate of DDVP from the strip, and duration of the activity. The durations will be taken as the same as in the dermal exposure scenario, a central estimate of 1 hour with a range of 0.25 to 4 hours. The initial release rate will be taken as 0.015 hour^{-1}. This is calculated

from the study by Slomka and Hine (1981) which indicated that approximately 30% of the DDVP was released in the first 24 hours – i.e., $k = -\ln(1-f)/t = \ln(1-0.3)/24$ hours $= 0.01486$ hour^{-1}.]. The proportion of the strip that might be in the mouth of the child will be taken as 0.25 – i.e., a area of about 1 square inch. As indicated in Worksheet B02, this exposure assessment results in estimates of absorbed doses of about 0.2 mg/kg with a range of 0.04 mg/kg to 0.6 mg/kg. This scenario would also involve some dermal exposure. As indicated in Section 3.4, any plausible dermal exposure would likely be much less than the oral exposure and would have no impact on the characterization of risk.

3.2.3.4. Oral Exposure to Contaminated Water – Under normal circumstances, the use of DDVP in PVC strips is not likely to result in contamination of water or other materials that might be consumed by members of the general public. In the recent risk assessment by U.S. EPA (2000a), no exposure assessment for water contamination by DDVP in PVC formulations is presented.

The approach taken by U.S. EPA (2000a) seems reasonable in that the slow release DDVP from the test strip and rapid hydrolysis of DDVP in water is likely to limit the concentration of DDVP in ambient water. For example, the halftimes for the hydrolysis of DDVP in water range from about 11.65 days at pH 5 to 0.88 days at pH 9, with a hydrolysis halftime of 5.19 days at pH 7 (U.S. EPA 1999a, p. 3). These values correspond to hydrolysis rates – i.e., $k = \ln(2)/t_{50}$ – of 0.06 day^{-1} [pH 5], 0.13 day^{-1} [pH 7], and 0.78 day^{-1} [pH 9]. All of these hydrolysis rates are more rapid than the release rate of DDVP in air from the Hercon pest strip – i.e., 0.04 day^{-1} as discussed in Section 3.2.2.2.

For this risk assessment, the assumption will be made that a VaporTape strip accidentally contaminates a small pond (e.g., it is inadvertently dropped into a pond during placement of a trap or a trap is dislodged and falls or is blown into a pond). No data are available to directly estimate the amount of DDVP that might be released over the course of a single day. For this exposure assessment, the assumption will be made that 30% of the DDVP in a fresh strip might be released over the course of a single day. This is based on the study by Slomka and Hine (1981), discussed in Section 3.1.4, in which 30% of the DDVP was released from a pest strip into gastric juices over a 24 hour period. Thus, the central estimate of the amount of DDVP in water is taken as 177 mg [590 mg × 0.3]. The upper range of the amount of DDVP in water is taken simply as the amount of DDVP in a new pest strip – 590 mg. The selection of a lower is somewhat arbitrary and a value of 10% or 59 mg is used. Other details of this exposure assessment are given in Worksheet B03 and involve standard assumptions concerning the size of the pond and the amount of water that might be consumed. These assumptions are standard in risk assessments (SERA 2001). As detailed in Worksheet B02, dose estimates range from about 0.000003 mg/kg to 0.00007 mg/kg with a central estimate of about 0.00001 mg/kg.

As noted above, this very simple exposure scenario does not consider the degradation or dissipation of DDVP. As discussed further in Section 3.4, however, this exposure assessment leads to concentrations in water that are far below a level of concern. Thus, the overestimates of

concentrations in water developed in this section have no impact on the risk characterization for potential effects in humans.

3.3. DOSE-RESPONSE ASSESSMENT
3.3.1. Overview

The extensive toxicology data base has been evaluated by a number of governmental organizations including the U.S. EPA, the Agency for Toxic Substances and Disease Registry (ATSDR), National Institute for Occupational Safety and Health (NIOSH), Occupational Safety and Health Administration, and the World Health Organization. Following the approach taken in most USDA risk assessments, these sources are used for selecting levels of acceptable exposure. Because all of the scenarios considered in this risk assessment involve only acute exposures, only acute exposure criteria are considered.

For both oral and dermal exposures, the acute RfD established by the U.S. EPA, 0.0017 mg/kg, is used for the risk characterization. This is based on an acute oral NOAEL of 0.5 mg/kg from a study in rats with the application of an uncertainty factor of 300. Acute exposure criteria proposed by other groups are comparable to but somewhat higher than the acute RfD. Because some of the accidental acute exposures may substantially exceed the acute RfD, some attempt is made to characterize the consequences of high oral exposures. A human NOAEL of 1 mg/kg for AChE inhibition has been identified. While this NOAEL is not used to modify the acute RfD, it can be used to assess plausible consequences of exceeding the RfD. The human data on DDVP, although extensive, are not sufficient to identify a minimal lethal dose. For the current risk assessment, the lowest reported lethal dose (16 mg/kg) is used to assess the plausibility of observing serious adverse effects in cases of accidental over-exposure to DDVP.

A number of inhalation criteria for DDVP are available. Since potentially significant inhalation exposures are likely only in workers, the occupational exposure criterion of 0.1 mg/m^3 proposed by American Conference of Governmental Industrial Hygienists is used. This value is a factor of 10 below the occupational criteria proposed by NIOSH and OSHA.

3.3.2. Acute Exposures

3.3.2.1. Acute Oral – As summarized in Section 3.1.4, the U.S. EPA (2000a) bases the acute oral RfD for DDVP on the study by Bast et al. (1997) in which no effects, including assays for alterations in behavior, were noted at 0.5 mg/kg but neurological effects related to AChE inhibition were noted at 35 mg/kg. In deriving the acute RfD, the U.S. EPA (2000a, p. 18) used an uncertainty factor of 300 and recommended an acute RfD of 0.0017 mg/kg/day [0.5 mg/kg ÷ 300 = 0.0017 mg/kg]. ATSDR (1997) has recommended a somewhat higher acute oral minimal risk level (MRL) – a value that is analogous to the RfD – of 0.004 mg/kg/day. This is based on a 14-day LOAEL of 4 mg/kg/day in which brain AChE was inhibited by 44%. The MRL was calculated using an uncertainty factor of 1000 (ATSDR 1997, pp. 83-84).

As also discussed in Section 3.1.4, the study by Stanton et al. (1979) suggests that DDVP in a PVC formulation will be much less toxic than unformulated DDVP. The extent of the difference in toxicity, however, is difficult to quantify. For unformulated DDVP, the LD$_{50}$ value was 157 (113–227) mg/kg with no mortality observed at 56 mg/kg. For the DDVP-PVC formulation, no deaths occurred at doses of up to 1000 mg/kg, although signs of toxicity consistent with AChE

inhibition were observed at doses of 320 mg/kg and 1000 mg/kg using the DDVP-PVC formulation. No tremors or salivation were observed at doses of 240 or 180 mg/kg of the DDVP-PVC formulation. Stanton et al. (1979) do not provide comparative data the extent of AChE inhibition in unformulated DDVP and the DDVP-PVC formulation.

As detailed in Section 3.2.3.3, estimates of acute oral exposure for a small child sucking on a pest strip are far above the acute RfD of 0.0017 mg/kg. Thus, the potential for more severe effects must be considered. Based on the recent study by Gledhill (1997), no changes in AChE activity and no signs of toxicity were seen in a group of 6 men administered DDVP in a gelatin capsule at an approximate dose of 1 mg/kg. This is a factor of about 600 above the acute oral RfD. This study is unpublished and was submitted to the U.S. EPA by a registrant. In the U.S. EPA (2000a) human health risk assessment, the MRID number for this study is cited but the results of the study are not discussed specifically. For the current risk assessment, a dose of 1 mg/kg from the Gledhill (1997) study is used qualitatively to characterize the risks of exposures that are not likely to produce clinically significant effects.

For many pesticides, exposures that would be associated with severe and possibly fatal effects often can be estimated from poisoning reports. Most reports of fatal exposures to DDVP, however, do not provide sufficient information to estimate a lethal dose in humans. An approximate lethal dose, however, can be estimated from the study by Shimizu et al. (1996), which reports a fatal exposure of a 62.5 kg woman who intentionally consumed a pesticide formulation containing 75% DDVP and 25% xylene. While xylene is also a toxic agent, the oral LD_{50} for xylene in rodents is in the range of 3,500 to 8,600 mg/kg (ATSDR, 1995, p. 59). This is much greater than the reported LD_{50} values for DDVP in rodents – i.e., in the range of 25 to 300 mg/kg as summarized in Section 3.14. The amount of DDVP that the woman ingested is unclear. About 300 grams (300,000 mg) of DDVP were found in the stomach and Shimizu et al. (1996, p. 65) estimate that the woman probably absorbed about 1,000 mg/kg. Taking the estimated absorbed dose, a lethal dose for humans can be estimated at about 16 mg/kg [1,000 mg ÷ 62.5 kg]. This is not necessarily a minimum lethal dose – i.e., the individual might have died after ingesting a lesser amount of DDVP. Other reported poisoning cases involving DDVP (e.g., ATSDR 1997; WHO 1988) do not have sufficient information to estimate a minimum lethal dose for humans.

***3.3.2.2. Acute Dermal* –** For short-term dermal exposure, the U.S. EPA (2000a) recommends an oral NOAEL of 0.1 mg/kg with a margin of exposure of 300 for residential exposure and 100 for occupational exposure. This would correspond to an acute RfD of 0.00033 mg/kg for residential exposures and 0.001 mg/kg for occupational exposures. The U.S. EPA (2000a) recommends using this value with dermal deposition data and an assumed dermal absorption fraction of 11%.

These values will not be used in the current risk assessment. Following the general approach used in other risk assessments prepared for USDA (SERA 2001), the absorbed doses estimated in Section 3.2.2.3 for workers and Section 3.2.3.2 for the general public will be used with the acute oral RfD of 0.0017 mg/kg/day. The general rationale for this approach is given in SERA (2001).

For DDVP in particular, the standard approach used in USDA risk assessments is necessary because the incidental or accidental handling of VaporTape strips does lead to estimates of dermal deposition.

3.3.2.3. Acute Inhalation – For short-term inhalation exposures, the U.S. EPA (2000a) recommends the same acute toxicity value used for dermal exposures. Given the extensive inhalation toxicity data available for DDVP, the rationale for this approach is unclear. The U.S. EPA (1994) has derived an inhalation RfC for DDVP of 0.0005 mg/m^3. This is based on an animal NOAEL of 0.05 mg/m^3 with a corresponding LOAEL of 0.48 mg/m^3 from a two year exposure study in rats. As noted below, this chronic RfD is not relevant to the current risk assessment because no chronic exposures are anticipated. In addition to this value recommended by EPA, ATSDR (1997) has recommended an acute minimum risk level (MRL) of 0.002 ppm for DDVP which corresponds to a concentration of about 0.018 mg/m^3 – i.e., 1 ppm = 9.04 mg/m^3. This value is intended to be applied to exposure periods of up to 14 days.

As detailed in Section 3.2.2.2, all exposures for workers are short-term. OSHA and NIOSH share responsibility for proposing exposure criteria to protect workers. OSHA provides regulatory enforcement (exposure standards) and NIOSH provides science based exposure criteria (NIOSH 2002). For DDVP, NIOSH recommends a time-weighted average exposure limit of 1 mg/m^3 and this value has been adopted by OSHA (NIOSH 2002). Another group involved in recommending criteria for occupational exposure is ACGIH (2004), which recommended a lower occupational exposure limit of 0.1 mg/m^3 (ACGIH 1991). This lower value appears to have been selected by ACGIH (1991) based on an unpublished report to the TLV committee that exposures to 1 mg/m^3 over the course of a workday resulted in an inhibition of plasma AChE of 20%-25% in a group of workers (ACGIH 1991, p. 446). The documentation for the TLV, however, does not suggest that any adverse health effects were observed. The lower and more protective value of 0.1 mg/m^3 is adopted in the current risk assessment for the protection of workers during inhalation exposures.

3.3.3. Chronic Exposures

The U.S. EPA (2002), ATSDR (1997), and WHO (1998) have all recommended various criteria for chronic exposure to DDVP by oral, dermal, and/or inhalation routes. Because none of the exposure scenarios in this risk assessment involve chronic or subchronic exposures, these recommendations are not considered in the current risk assessment. While the previous USDA risk assessment (USDA 1995a) considered the potential cancer risks associated with exposure to DDVP, this approach is not adopted in the current risk assessment. As discussed in Section 3.1.10, the recent re-evaluation of the cancer data on DDVP (U.S. EPA 2000a,b) has concluded that the data available on the carcinogenicity of DDVP is not sufficient for quantitative risk assessment.

3.4. RISK CHARACTERIZATION
3.4.1. Overview
The quantitative risk characterizations for workers and members of the general public are summarized in Table 3-3. This table is taken directly from Worksheet C02 and is included in the body of the risk assessment only for convenience.

In most cases, exposures to both workers and members of the general public should be negligible. If workers take prudent steps to limit both dermal and inhalation exposures, the likelihood of exposures to DDVP reaching a level of concern appears to be very low. Similarly, members of the general public should not be exposed to substantial amounts of DDVP. The DDVP is contained within a PVC strip to insure that the active ingredient is slowly released over a long period of time. The strip, in turn, is placed within a trap and the trap is placed in areas that will not be generally accessed except in the case of intentional tampering or trap monitoring.

Nonetheless, this risk assessment develops exposure scenarios for both workers and members of the general pubic that are intended to illustrate the potential effects of mishandling or tampering with DDVP strips. For workers, the greatest risks are associated with inhalation exposures from assembling the traps in enclosed and poorly ventilated spaces or transporting the traps in the passenger compartments of vehicles. These risks can be readily avoided. Dermal exposures can also lead to lesser but sill undesirable levels of exposure. For members of the general public, all of the exposure scenarios are accidental and some are extreme. The most likely of these is the accidental contamination of a small body of water. This scenario leads to exposures that are below the level of concern by a factor of about 25. If a child were to come into contact with a DDVP strip, however, both dermal and oral exposures could substantially exceed a level of concern. While such exposures should clearly be avoided, it does not seem likely that frank signs of toxicity would be observed. This is consistent with human experience in the use of DDVP resin strips.

3.4.2. Workers
The risk characterization for workers is highly dependant on how the worker handles the DDVP strip during assembly of the milk carton trap. If the trap is assembled outdoors and if the worker wears protective gloves during the assembly of the trap, both dermal and inhalation exposures as well as consequent risk should be negligible. Whether or not this is common practice is unclear. The MSDS states that gloves (vinyl, latex, or rubber) should be worn if the strip is handled for prolonged periods of time (Hercon 1993). The product label (Hercon 2004) indicates that hands should be washed thoroughly after handling the pest strip. In addition, the Gypsy Moth Program Manual (USDA 2001, p. E-6) recommends that workers "*use the outer package or rubber gloves to handle the insecticide strip. Handle the insecticide strip as little as possible*". If these recommendations are followed, direct dermal exposure to DDVP should be negligible.

If workers assemble traps in enclosed areas or do not use protective gloves during the assembly of traps or take other measures to prevent dermal exposure, it is plausible that exposures will exceed a level of concern. As summarized in Table 3-3, the potential for undesirable inhalation

exposures is substantial – i.e., risk quotients up to 18 – if the traps are assembled or transported in areas with poor or no ventilation. As discussed in Section 3.2.2.2 and detailed further in Appendix 1, these exposure assessments are based on a large number of site and situation specific factors – i.e., the volume of the room or area in which the strips are assembled or transported, the number of strips that are involved, and the ventilation rates of the area in which exposure occurs. Thus, if the pest strips are assembled indoors, it would be prudent to modify Worksheet A03a and ensure that the local conditions would likely lead to air concentrations that are below the ACGIH (1991) TLV of 0.1 mg/m^3.

It should be noted that the risk quotients associated with transport of the pest strips in the passenger compartment of a vehicle are substantially higher than risk quotients during assembly of the traps in a room. High ventilation rates – i.e., 3000 air turnovers per day or about 2 air turnovers per minute as detailed in Worksheet A03b – could probably be achieved in a vehicle by rolling down the window and this would reduce the inhalation exposure to below the level of concern. Nonetheless, transporting DDVP or any volatile neurotoxic agent in the passenger compartment of a vehicle is clearly imprudent and should be avoided.

Dermal exposure is of lesser and only modest concern based on the exposure assessments. As noted in Table 3-3, the acute RfD is modestly exceeded – i.e., a hazard quotient of 3 – at the upper range of estimated exposures if workers do not wear gloves . This risk quotient is associated with a dose of about 0.005 mg/kg bw. It seems unlikely that any adverse effects would be experienced at this dose level, which is a factor of 200 below the human NOAEL of 1 mg/kg [1 mg/kg ÷ 0.005 mg/kg = 200] and a factor of 3,200 below the lowest reported lethal dose in humans [16 mg/kg ÷ 0.005 mg/kg = 3200]. While there are uncertainties with the exposure assessment on which the risk quotient of 3 is based, contamination of the skin in workers not wearing gloves seems to be highly likely. As noted in the product label for the VaporTape II strip: "*After prolonged storage, a small amount of liquid may form on the strip*" (Hercon 2004). This liquid would presumably contain DDVP which would contaminate the surface of the exposed skin. It is also worth noting that the exposure assessment assumes that only the tips of the fingers are contaminated and that the duration of exposure is only 15 minutes to 1 hour. If the worker were to contaminate a greater area of the skin or to spend a longer period of time assembling the traps, the estimated doses would be greater.

3.4.3. General Public
The nature of risks to the general public is substantially different from those to workers. As detailed in the previous section, undesirable levels of exposure are plausible for workers if sensible measures are not taken to limit exposure. For members of the general public, essentially no significant exposures are plausible. The accidental contamination of a small pond with a pest strip (Worksheet B02) is probably the most likely exposure scenario. As indicated in Table 3-3, this exposure scenario leads to levels of risk that a very low – i.e., the highest hazard quotient is 0.04, below the level of concern by a factor of 25.

The probability of a child tampering with a trap is low because the traps will not generally be placed in areas that the general public will frequent and will be placed so that the traps are not easily accessible to children. Thus, the exposure scenarios involving a child either tampering with a trap or otherwise coming into direct contact with a DDVP strip appear to be highly unlikely. As illustrated in Table 3-3, dermal exposures would lead to risk quotients of up to 60. These exposures would be associated with doses of up to about 0.1 mg/kg (Worksheet B01b). This dose is below the lowest reported lethal dose in humans by a factor of about 160 [16 mg/kg ÷ 0.1 mg/kg], below the acute human NOAEL of 1 mg/kg by a factor of 10, and below the acute animal NOAEL of 0.5 mg/kg by a factor 5. Thus, while this type of exposure would be considered unacceptable, the plausibility of observing toxic effects seems remote.

The plausibility and consequences of oral exposures for a child tampering with a DDVP strip are very difficult to assess. The unpleasant taste and smell of the pest strip should help to decrease the amount of exposure; however, there are reported cases of child poisoning by pest strips containing DDVP, although none of the exposures have been fatal. Nonetheless, the oral exposure scenarios developed in this risk assessment lead to the highest risk quotients for DDVP, a central estimate of 97 with a range of 24 to 380 (Table 3-3 and Worksheet C02). These risk quotients are associated with doses of about 0.2 mg/kg with a range of about 0.04 mg/k to 0.6 mg/kg. As with the dermal exposures for a small child, these exposures should be clearly regarded as unacceptable. Nonetheless, it is not clear that any significant adverse effects would be observed since the dose estimates are below the human NOAEL of 1 mg/kg and the upper range of exposure is below the lowest reported lethal dose by a factor of over 25 [16 mg/kg ÷ 0.6 = 26.7]. Thus, while these exposure scenarios may be considered extreme and could warrant prompt medical attention as a precautionary measure, it is possible that no serious adverse effects would be observed. This risk characterization is consistent with the assessment of incidents involving exposures to DDVP resin strips – *"exposure to resin strips usually do not involve any significant acute symptoms that would require medical treatment"* (U.S. EPA 2000a, p. 26).

3.4.4. Sensitive Subgroups
Children are of primary concern to this risk assessment. As noted above, imprudent handling of a DDVP impregnated strip would most likely involve a child. In addition, very young children (that is, infants less than 6-months old) may be at special risk because they have incompletely developed AChE systems and immature livers (ATSDR 1993). Several other groups may be at special risk to all cholinesterase inhibiting compounds, including DDVP. A small proportion of the population has an atypical variant of plasma cholinesterase. This condition is known to make these individuals sensitive to succinylcholine and may make them more susceptible to exposure to DDVP and other AChE inhibitors. Other groups known to have low plasma AChE levels are long-distance runners, women in early stages of pregnancy, women using birth control pills, individuals with advanced liver disease, alcoholics, individuals with poor nutritional status, and individuals with skin diseases. Asthmatics may also be at special risk because DDVP may induce or exacerbate respiratory distress (ATSDR 1993).

3.4.5. Connected Actions

There are no data regarding the effects of exposure to DDVP combined with exposure to the other agents used to control the gypsy moth or the gypsy moth itself. Inhibition of AChE is the most sensitive effect of DDVP. This effect is not associated with exposure to the other control agents or exposure to the gypsy moth. Therefore, there is no plausible basis for assuming that the effects of exposure to DDVP and any or all of the other control agents or the gypsy moth will be additive.

Exposure to other compounds that inhibit AChE are likely to lead to an additive effect with DDVP. The most common examples include any other organophosphate or carbamate pesticides (ATSDR 1993; Gallo and Lawryk 1991). Thus, if members of the general public or workers use other organophosphate pesticides to the extent that AChE activity is substantially inhibited, they could be at increased risk if exposed to significant levels of DDVP.

No studies were located regarding toxicological interactions between Vaportape II and other chemicals. There are several studies regarding combined exposures to commercial grade DDVP and other chemicals, all of which involve animal exposure, and, in most cases, overtly neurotoxic doses of DDVP administered by acute injections. Of the few studies regarding oral or dermal exposure to DDVP, most involve acute durations of exposure and do not provide adequate evidence of toxicological interactions. Nevertheless, some of these studies are discussed here because they concern certain interactions that are generally associated with organophosphate insecticides as a class and because they are relevant to the issue of whether or not such interactions involving DDVP are plausible.

Phenothiazine-derived drugs such as chlorpromazine have been shown to enhance the toxicity of acutely administered organophosphate insecticides such as parathion (Calabrese 1991). The mechanism for this enhancement is not known and may involve altered metabolic activation or deactivation of the organophosphate. The interaction between topically applied DDVP/Crotoxyphos insecticide and orally administered phenothiazine anthelmintic has been studied to a limited extent in livestock, and no obvious interactions have been observed. A series of case studies were reported in which young cattle were treated with topical doses of various organophosphate insecticides at the end of a 30-day oral treatment with phenothiazine anthelmintic, followed by DDVP/Crotoxyphos insecticide 1 month later. There was no evidence of an interaction between the phenothiazine and DDVP/Crotoxyphos insecticide (Schlinke and Palmer 1973). In a more controlled study, lambs were treated orally with phenothiazine antihelmentic (12.5 g initially and 4 days later with 6.25 g every 3 days for nine treatments) or topical application of an emulsifiable mixture of 2.3% DDVP and 10% Crotoxyphos (1,550 mL of 0.25% emulsion sprayed every 2 weeks for three applications) or both. Erythrocyte acetylcholinesterase inhibition and clinical signs of acetylcholinesterase inhibition occurred within 40 minutes after each DDVP/Crotoxyphos mixture spray; the severity of the effects was not affected by the concurrent phenothiazine treatment (Mohammad and St. Omer 1983, 1985).

Because of their ability to inhibit acetylcholinesterase and thereby alter the metabolism and deactivation of acetylcholine, organophosphate insecticides are expected to interact with drugs that mimic the effect of acetylcholine (cholinergic drugs) or that block the effects of acetylcholine (anticholinergic drugs). In fact, the anticholinergic drug, atropine, is indicated for treatment of severe cholinergic symptoms of organophosphate insecticide toxicity. Because both cholinergic and anticholinergic drugs have many other uses, inadvertent interactions in which the organophosphate insecticide alters the effect of the drug also should be considered. Acute interactions of this type involving DDVP have been studied only to a limited extent in animal models of peripheral cholinergic control mechanisms. In one such study, the anticholinergic drug, atropine, was administered to dogs (0.022 mg/kg by intramuscular injection) 90 minutes after an acute oral dose of 60 mg/kg DDVP, and the heart rate was monitored for cholinergic (decreased rate) and anticholinergic (increased rate) effects. Although the DDVP dose alone had no effect on heart rate, it did attenuate the acceleration of the heart rate caused by atropine. The DDVP dose decreased plasma and erythrocyte cholinesterase by approximately 50% (Dellinger et al. 1987). This study suggests that interactions in which DDVP affects the actions of anticholinergic drugs (for example, atropine, scopolamine, belladonna alkaloids) are plausible; however, there is no evidence of such interactions in humans.

Chemicals that inhibit carboxyesterases such as the non-organophosphate insecticide, triorthotolyl phosphate (TOTP), have been shown to enhance the toxicity of certain organophosphate insecticides. Inhibition of carboxyesterases may be a mechanism by which certain organophosphate insecticides act synergistically (Calabrese 1991). The significance of this interaction mechanism to DDVP toxicity has not been thoroughly investigated. In a study using mice, an acute intraperitoneal dose of TOTP 3 days before DDVP treatment enhanced the toxicity of an acute intraperitoneal dose of either malaoxon or paraoxon but did not alter the toxicity of an intraperitoneal dose of DDVP. Dieldrin, administered orally 4 days before sacrifice, increased liver carboxyesterase activity but had no effect on the toxicity of subsequently administered DDVP (Ehrich and Cohen 1977). This study suggests that carboxyesterase inhibitors may have a more significant effect on malaoxon and paraoxon toxicity than on DDVP toxicity.

The interaction of DDVP with other commonly occurring chemicals in the environment has not been well studied. In rats, pretreatment with acetaminophen, a common analgesic, had no effect on the acute toxicity of DDVP (Costa and Murphy 1984).

Toxicological interactions of DDVP have not been studied extensively or well enough to be of use in quantitative risk assessment. The few studies described here suggest that certain interactions typical of the organophosphate insecticides as a class (for example, anticholinergic agents) are plausible for DDVP. Nevertheless, there is no evidence that such interactions actually occur in humans. Furthermore, the studies regarding those kinds of interactions in animals have examined single exposures and have focused only on the acute anticholinesterase activity as the toxic endpoint (usually assessed by measurements of plasma or blood cholinesterase or cholinergic symptoms). There need to be more complete interaction bioassays

that examine multiple dose levels and durations, and more complete assessments of toxicity if risks related to possible interactions are to be assessed.

3.4.6. Cumulative Effects
Cumulative effects associated with DDVP exposures might be associated with repeated exposures during a single season or repeated exposures over several seasons. For the general public, the only substantial exposures will occur from tampering with traps containing DDVP. Such incidents have not been reported despite the long use of DDVP in traps for the gypsy moth as well as other species. These scenarios are considered in this risk assessment as accidental exposures, which occur infrequently. Consequently, it does not seem reasonable to expect that the same person will be involved repeatedly in such unusual exposures.

Workers, on the other hand, may be exposed repeatedly to DDVP if they are involved in the assembly and placement of traps over a period of several weeks. Such exposures, however, are encompassed by the current risk assessment. For inhalation exposures, the risk is characterized using the TLV (ACGIH 1991). The TLV is intended to be protective of exposures that occur during a typical career (for example, 8 hours/day, 5 days/week, for 20 years).

For some organophosphates, concern about cumulative effects is diminished because studies have demonstrated tolerance to repeated exposures (Gallo and Lawryk 1991). This tolerance has not been demonstrated for exposure to DDVP. As is true for exposures involving the general public, concern for repeated exposures is diminished because, under normal handling conditions, substantial levels of exposure are not anticipated.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION
4.1.1. Overview
As described in Section 3.1.2., DDVP is an organophosphate insecticide. DDVP inhibits acetylcholinesterase (AChE) activity, resulting in overstimulation of cholinergic neurons. Inhibition of this enzyme in mammalian systems produces a variety of systemic effects, including salivation, urination, lacrimation, convulsions, increased bronchial secretions, respiratory depression, and even death. DDVP is readily absorbed by the oral, dermal, and inhalation routes of exposure. Because the target enzyme (cholinesterase) for DDVP is common to mammals, fish, fowl, and insects, toxicity due to DDVP exposure can result in all of these species. By contrast, DDVP exhibits low toxicity to plants.

The available data suggest that invertebrates are more sensitive to DDVP than other organisms. For example, the oral LD_{50} in honey bees is 0.29 ì g/g bee, and the topical LD_{50} is 0.65 ì g/g bee. DDVP is also toxic to birds with an oral LD_{50} value of < 10 mg/kg for the most sensitive species. Short-term repeat dose studies in mammals found that oral exposures to doses below about 0.5 mg/kg-day or inhalation exposures to 1–2 mg/m³ generally do not result in adverse effects.

Aquatic animals are also sensitive to DDVP and, as with terrestrial animals, invertebrates may be more sensitive than vertebrates. The lowest reported LC_{50} value in fish is approximately 0.2 mg/L. Some aquatic invertebrates are much more sensitive to DDVP than fish. For daphnids, the most sensitive group of invertebrate species, reported EC_{50} values range from 0.00007 mg/L to 0.00028 mg/L.

The majority of the toxicity data in ecological receptors is limited to free DDVP, rather than a slow-release formulation such as the Vaportape II product used in USDA programs for control of the gypsy moth. Hence, the toxicity values reported for indicator species will likely be conservative (i.e., suggest greater toxicity) as compared to Vaportape II. U.S. EPA has assessed the ecological effects of DDVP; however, the exposures assessed by U.S. EPA are not specific to formulations where DDVP is encapsulated in PVC resin. In general, aside from those organisms that enter the milk carton trap or those that remove the strip from the trap, toxicity resulting from exposure of ecological receptors to DDVP in Vaportape II milk carton traps is not likely.

4.1.2. Toxicity to Terrestrial Organisms.
4.1.2.1. Mammals – As summarized in Section 3.1, the database includes a number of toxicity studies in experimental mammals. The principal adverse effects of DDVP exposure are directly related to inhibition of cholinesterase (the mode of action for DDVP). Inhibition of this enzyme in mammalian systems produces a variety of systemic effects (Table 3-1). The nature and magnitude of the toxicity produced by a given exposure to DDVP by any route are directly related to the dose and rate at which the exposure occurs. In USDA programs for the control of the gypsy moth, the use of milk carton traps employing slow-release of DDVP from PVC strips essentially precludes rapid exposures to high doses of DDVP. As described in Section 3.1.4,

short-term animal studies have shown that oral exposures to free DDVP below about 0.5 mg/kg-day (or inhalation exposures to 1–2 mg/m³) do not result in meaningful reductions in cholinesterase activity. Experiments in laboratory mammals that were exposed to DDVP during pregnancy (by oral or inhalation route) did not show any effect on fertility or health of the offspring, even at levels that produced maternal toxicity (see Section 3.1.9).

Dietary administration of DDVP (free and encapsulated in PVC resin pellets) has been used as a veterinary anthelminthic agent in a variety of species, including dogs (Batte et al. 1966; Batte et al. 1967), pigs (Batte et al. 1965; Bris et al. 1968; Stanton et al. 1979; Todd 1967), horses (Himes et al. 1967), sheep (Bris et al. 1968), cattle (Bris et al. 1968), dromedary camels (Wallach and Frueh 1968), and non-human primates (Wallach and Frueh 1968). In general, oral administration of DDVP produced no signs of organophosphate poisoning at doses that were effective at reducing intestinal parasites (Wallach and Frueh 1968). For example, two consecutive days of dosing at 2.3 in camels or 1.7 mg/kg in non-human primates, respectively, was well tolerated by the animals despite debilitating intestinal infection (Wallach and Fueh 1968). In cows, Lloyd and Matthysse (1971) reported that diets containing DDVP (in PVC pellets) at doses 1.3, 1.8, or 2.3 mg/kg bw for 14 days produced no adverse effect on milk production (no other effects were reported). No DDVP was found in the milk at 1, 3, 7, 10 or 14 days. Free DDVP – i.e., not encapsulated in a PVC resin – produced severe inhibition of cholinesterase activity at a dose of 4.5 mg/kg (Tracey et al 1960).

As discussed in Section 3.1.4, the effect of PVC encapsulation on the toxicity of DDVP has been quantified in parallel assays (Stanton et al. 1979), in which DDVP (undiluted technical grade) and DDVP (impregnated in PVC) were administered to groups of young swine. For the technical grade liquid formulation, the LD_{50} was 157 (113–227) mg/kg and the NOAEL based on lethality was 56 mg/kg. For the PVC formulation, no deaths occurred at any doses including 1,000 mg/kg, the highest dose tested.

As discussed in Section 3.1.16, simultaneous exposure to DDVP and another cholinesterase inhibitor (e.g., organophosphate or carbamate insecticides) or a cholinomimetic agent (e.g., pilocarpine and carbachol) would likely enhance the cholinergic toxicity produced by DDVP. This is the major toxicologic interaction for DDVP. In addition, Short et al. (1971) also reported that DDVP exposure in combination with the muscle relaxant succinylcholine can produce cardiac arrythmias, apnea, and death in Shetland ponies depending on the degree of cholinesterase inhibition.

4.1.2.2. Birds – The acute oral LD_{50} in birds ranges from 6.5–24 mg/kg (WHO 1989, Hudson et al. 1984, Grimes and Aber 1988). As in mammals, the signs of DDVP intoxication in birds are typical of organophosphorus poisoning (e.g., tremors, and convulsions) and usually appear shortly after dosing. At lethal doses, death occurs within 1 hour, with survivors recovering completely within 24 h after dosing (WHO 1989). Tucker and Crabtree (1970) found various internal hemorrhages at autopsy in sacrificed pheasants and mallard ducks that survived acute high dose exposures.

The data from unpublished egg production and hatchability studies suggests that mallard ducks are more sensitive to DDVP than northern bobwhite quail. In mallard ducks, 20 weeks of dietary exposure identified a NOEC of 5 ppm and a LOAEL of 15 ppm based on number of eggs laid, eggshell thickness, number of viable embryos and number of live 3-week embryos (Redgrave and Mansell 1997). Cameron (1996) reported no effect on bobwhite quail reproduction following dietary exposure to DDVP at concentrations of 12 or 30 ppm for 20 weeks. At 100 ppm, however, statistically significant reductions in the number of eggs laid, viable embryos, live 3-week embryos, and survivors at 14 days. The short-term dietary LD_{50} in birds (5 days of exposure followed by three days of untreated diet) ranged from 300 ppm in Japanese quail to 5000 ppm in mallard ducks (Hill et al. 1975). Using chick and duck eggs, injections with DDVP at various incubation stages revealed that the LD_{50} values for these avian species at the mid-incubation stage were comparable to the rodent oral LD_{50} values (i.e., >50 mg/kg) (Khera and Lyon 1968).

Five days of continuous exposure of canaries, Indian finches, and budgerigars to DDVP vapor at 0.14 mg/m³ reduced cholinesterase activity, but produced no overt signs of organophosphate intoxication (Brown et al. 1968, as cited by WHO 1989).

It is important to note that the LD_{50} values reported from these studies are derived from the active ingredient, DDVP, in free form. Encapsulation in PVC resin (such as Vaportape II used in milk carton traps) would be expected to slow the release of DDVP, thereby reducing the acute toxicity and increasing the LD_{50} values (Section 3.1.4). No published data are available concerning the acute toxicity of DDVP encased in PVC resin in birds.

4.1.2.3. Terrestrial Invertebrates – In general DDVP is highly toxic to invertebrates with effect levels for honey bees below 1 ì g/g bee. In laboratory studies of honey bees, Atkins et al. (1973) found an LD_{50} of 0.495 ì g/bee in 48 h (topical application of dust; 26.7 °C with a relative humidity 65%). Beran (1979) reported an oral LD_{50} of 0.29 ì g/g body weight and a topical LD_{50} of 0.65 ì g/g body weight.

A variety of other studies are available; however, they are not reported in sufficient detail to provide quantitative estimates of exposures. Nevertheless, these studies support the conclusion that invertebrates are highly susceptible to the toxic effects of DDVP. Following the exposure of honeycombs to DDVP vapor emanating from DDVP resin strips for 4 months, the combs absorbed the insecticide and were toxic to bees for approximately one month after exposure. Contamination of the bees appeared to be by inhalation rather than direct contact (Clinch 1970). Consumption of mulberry leaves sprayed with 1.56–6.25 mg/L DDVP produced 50% mortality in silkworm larvae after 4 hours of feeding (Aratake and Kayamura 1973). No adverse effects were observed on the hatchability and general condition of silkworm larvae hatched in the generation following feeding of mulberry leaves pre-treated with 3 mg/kg DDVP of leaf to adults (Yamanoi 1980).

4.1.2.4. Terrestrial Plants (Macrophytes) – Neither the published literature nor the review documents include data regarding the phytotoxicity of DDVP. Given the mode of action of DDVP, the U.S. EPA (1999a) has determined that toxicity testing in plants is not required for registration.

4.1.2.5. Terrestrial Microorganisms – WHO (1989) reported that the effect of DDVP on microorganisms is variable and species dependent. Certain microorganisms are able to metabolize DDVP, but DDVP may interfere with the endogenous oxidative metabolism of the organism. In certain organisms DDVP inhibits growth, while in others it has no influence or may stimulate growth. The above effects have been seen over a concentration range of 0.1–100 mg/L (Lieberman and Alexander 1981).

As noted earlier, the LD_{50} values reported from these studies are derived from the active ingredient, DDVP, in free form. Encapsulation in PVC resin (such as Vaportape II used by the Forest Service in milk carton traps) would be expected to slow the release of DDVP, thereby reducing the acute toxicity and increasing the LD_{50} values. No published data are available concerning the acute toxicity of DDVP encased in PVC resin in terrestrial microorganisms.

4.1.2.6. Terrestrial Field Studies – No terrestrial field studies on the effects of free DDVP or DDVP in PVC resin were located. Whitehead (1971) has advised caution in the use and handling of DDVP, where birds might be exposed because of their particular sensitivity to the toxic effects of organophosphate poisoning.   In the case of the USDA programs involving the use of DDVP in traps, however, the probability of widespread contamination of soil or aquatic ecosystems is very low because a small amount of DDVP (590 mg) is used in the Vaportape II trap and because the DDVP is released slowly from the PVC resin.

4.1.3. Aquatic Organisms.
4.1.3.1. Fish – DDVP is classified as highly toxic to both freshwater and estuarine fish (U.S. EPA 1999a). In freshwater fish, reported 96-h LC_{50} values range from about 0.2 mg/L for lake trout or cutthroat trout and 12 mg/L for fathead minnows (U.S. EPA 1999a, p. 12). In estuarine fish, 96-h LC_{50} values range from 0.23–14.4 mg/L for striped mullet and mummichog, respectively (U.S. EPA 1999a, p. 12). Sublethal effects – i.e., brain and liver cholinesterase inhibition – have been reported in fish at doses of 0.25–1.25 mg/L, but cholinesterase activity recovered when the fish were returned to clean water (WHO 1989). The acute toxicity of DDVP in cutthroat trout or lake trout was not altered by variations in water hardness from 44 to 162 mg/L or at pH 6 to 9 (Johnson and Finley 1980).

Studies of sublethal effects in fish, most involving exposure periods of about 30 days, have demonstrated that exposure to ≤1 mg/L DDVP may produce changes in respiratory rates, serum and liver enzyme activity (aside from cholinesterase), lipid and carbohydrate metabolism, and hemoglobin and clotting time (WHO 1989). From these reports of adverse effects in fish, WHO (1989) derived an NOEC of 0.03 mg/L.

Only unpublished studies submitted to U.S. EPA were located regarding the chronic toxicity of DDVP in fish. These studies are all summarized in U.S. EPA (1999a). A NOEC of 0.0052 mg/L was reported for rainbow trout with a corresponding LOAEL of 0.0101 mg/L for a reduction in larval survival. Another study found that 0.96 mg/L produced no effects on fry of sheepshead minnow, whereas 1.84 mg/L produced statistically significant reductions in fry survival and length. As discussed in Section 3.1.7., *in vitro* studies on cells from embryonic renal tissue of carp demonstrated a dose-related decrease in lymphocyte proliferation and myeloid cell respiratory burst activities, both of which indicate immunosuppression; however, no effects on antibody production were noted in an *in vivo* study of carp cells (Dunier et al. 1991). The authors concluded that the results suggest that chronic exposure to DDVP may impair the immune system of fish.

4.1.3.2. Amphibians – Neither the published literature nor the review documents include data regarding the toxicity of DDVP to amphibians.

4.1.3.3. Aquatic Invertebrates – In general, invertebrates tend to be more sensitive to the toxic effects of DDVP than fish. Whereas the lowest reported LC_{50} value reported in fish is 0.183 mg/L (the value for lake trout reported by U.S. EPA 1999a, p. 12), the lowest comparable value reported for aquatic invertebrates is 0.00007 mg/L (the 48-hour EC_{50} value for *Daphnia pulex* reported by U.S. EPA 1999a, p. 13). Based on these measures, aquatic invertebrates appear to be more sensitive than fish by a factor of over 2500 [0.183 mg/L ÷ 0.00007 mg/L = 2614]. WHO (1989) reports that the acute toxicity of DDVP to aquatic insects (stone fly) and estuarine crustaceans (hermit crab) is also extremely high (96-hour LC_{50} values ranging from 0.0001–0.045 mg/L, respectively).

As with the data on fish, some of the more important studies are unpublished and have been submitted to U.S. EPA for the registration of various uses of DDVP (U.S. EPA 1999a). As summarized by U.S. EPA (1999a), the 48-hour EC_{50} values in two species of water flea range from 0.00007 mg/L to 0.00028 mg/L. In an unpublished 21-day study in daphnids, the NOEC and LOEC are 0.0000058 mg/L and 0.0000122 mg/L, respectively.

Not all species of aquatic invertebrates, however, are this sensitive. The most remarkable exception to the sensitivity of aquatic invertebrates to DDVP is the freshwater snail; Jonnalagadda and Rao (1996) reported a 96-hour LC_{50} of approximately 21 mg/L in this species. Exposure of prawns to DDVP concentrations of 0.31 or 0.62 mg/L for 96 hours produced a decrease in hepatic glycogen and an increase in the blood glucose level (Omkar and Shukla 1984).

Forget et al. (1998) report static 96-hour LC_{50} values for copepods ranging from 0.00092–0.0046 mg/L (different sensitivity depending on life stage). Treatment of eutrophic carp ponds with 0.325 mg/L DDVP killed *Cladocera* (predominantly *Bosmina* and *Daphnia* species) and decreased cyclopods (mainly *Cyclops*). These reductions were offset by increased development of rotifers (mainly *Polyarthra* and *Brachionus* species) and phytoplankton (mainly *Scenedesmus*

and *Pediastrum* species), so that the total plankton biomass changed only slightly (Grahl et al. 1981).

4.1.3.4. Aquatic Plants – The database for DDVP does not contain many reports of its toxicity in aquatic plants. In an unpublished report cited by U.S. EPA 1999a), EC_{50} values >100 ppm are reported for green algae, 14 ppm for algae (NOS), and 17-28 ppm for marine diatoms. Butler (1977) reported that 3.5 mg/L DDVP produces 50% growth inhibition of *Euglena gracilis* (algae).

4.1.3.5. Other Aquatic Microorganisms – Neither the published literature nor the review documents include data regarding the toxicity of DDVP to other aquatic microorganisms.

4.2. EXPOSURE ASSESSMENT
4.2.1. Overview
As in the human health risk assessment, exposure of terrestrial mammals to DDVP from the VaporTape strips used in milk carton traps is likely to be negligible under most circumstances. Nonetheless, it is conceivable that some mammals such as racoons or bears could easily access and tamper with the milk carton trap. Depending on the proportion of the DDVP strip that is consumed, doses (as DDVP in the PVC strip) are estimated to range from 10.5 mg/kg (10% of strip) to 105 mg/kg (100% of strip) and the central estimate is taken as 31.6 mg/kg (30% of strip). In addition, contamination of water with a pest strip is plausible, although probably rare, and is considered in a manner similar to the corresponding scenario in the human health risk assessment (Section 3.2.3.4). This scenario is based on the consumption of contaminated water by a small mammal and the dose to the animal is estimated at about 0.00003 mg/kg with a range of 0.000009 mg/kg to 0.00009 mg/kg. Other exposure scenarios for terrestrial vertebrates, while possible, seem far less plausible and are not considered quantitatively. No quantitative exposure assessments for terrestrial invertebrates are developed because the milk carton trap will attract only male gypsy moths because of the pheromone bait in the milk carton trap. Nontarget insects that incidentally enter the trap are likely to be killed by exposure to the DDVP vapor. Exposures to aquatic species are based on the same water concentrations used for terrestrial species: 0.000177 mg/L with a range of 0.000059 mg/L to 0.00059 mg/L.

4.2.2. Terrestrial Vertebrates
4.2.2.1. Oral Exposure to DDVP Strip – For the exposure of a young child discussed in Section 3.2.3.3, only sucking on the strip rather than ingestion of all or part of the strip is considered. Various species of wildlife, however, are probably capable of consuming all or part of a pest strip. For the current risk assessment, it will be assumed that a racoon tampers with a milk carton trap and consumes part or all of the strip – i.e., 590 mg of DDVP in the PVC formulation. Taking a body weight of about 5.6 kg for an adult racoon (the average of the values reported by U.S. EPA/ORD 1993, p. 2-236) and assuming that the animal consumes between 10% and 100% of the strip with a central value of 30%, the dose to the racoon would be about 31.6 mg/kg with a range of 10.5 mg/kg to 105 mg/kg (Worksheet D01).

4.2.2.2. Oral Exposure to Water Contaminated with DDVP – Estimated concentrations of DDVP in water are identical to those used in the human health risk assessment (Worksheet B02) and involve the accidental contamination of a small pond with a DDVP-PVC strip. The only major differences in this scenario compared to the scenario in the human health risk assessment involve the weight of the animal and the amount of water consumed. There are well-established relationships between body weight and water consumption across a wide range of mammalian species (e.g., U.S. EPA/ORD 1993). These relationships are used to estimate the amount of water that a 20 g mammal would consume in one day (Worksheet D02). Unlike the human health risk assessment, estimates of the variability of water consumption are not available. Thus, for this acute scenario, the only factor affecting the variability of the ingested dose estimates is the amount of DDVP that might be released in one day. These amounts are discussed in Section

3.2.3.4 and are used in Worksheet D02. As indicated in Worksheet D02, the central estimate of the dose is about 0.00003 mg/kg with a range of 0.000009 mg/kg to 0.00009 mg/kg.

4.2.3. Terrestrial Invertebrates

As in the previous risk assessment (USDA 1995b), quantitative exposure assessments for terrestrial invertebrates are not considered. The only terrestrial invertebrates that are likely to come into close contact with the DDVP strip are male gypsy moths, which will be attracted by the disparlure in the trap, or carnivorous wasps and hornets that may enter the trap to feed on dead and dying gypsy moths. Other insects and perhaps other invertebrates such as spiders might incidentally enter the milk carton traps. Because DDVP in a non-specific insecticide, nontarget invertebrates would likely be killed by exposure to the DDVP vapor within the trap.

4.2.4. Aquatic Species

The exposure assessment for aquatic species is based on concentrations of DDVP in water that are identical to the concentrations used in the human health risk assessment (Worksheet B02) and the exposure assessment for a small mammal drinking contaminated water (Worksheet D02). As indicated in these worksheets, the central estimate of the concentration of DDVP in the pond is 0.000177 mg/L with a range of 0.000059 mg/L to 0.00059 mg/L.

4.3. DOSE-RESPONSE ASSESSMENT
4.3.1. Overview
Given the limited nature of the use of DDVP in programs to control the gypsy moth and
consequent limited number of exposure assessments, the dose-response assessment for DDVP is
relatively simple. For terrestrial mammals, a value of 240 mg/kg from a study using DDVP in a
PVC formulation is used for direct exposure to the DDVP-PVC strip – i.e., a raccoon tampering
with a milk carton trap and consuming all or part of the DDVP strip.   At the dose of 240 mg/kg,
no mortality or frank signs of AChE inhibition were observed. For the contaminated water
scenario, the NOAEL of 0.5 mg/kg from a study involving exposure to free or unformulated
DDVP is used. This NOAEL is from the study that forms the basis for the acute RfD used in the
human health risk assessment. Although DDVP is classified as highly toxic to fish, the estimated
levels of acute exposure for fish are far below the 30-day NOEC of 0.03 mg/L. Thus, this value
is used for all fish and no attempt is made to consider differences in sensitivity among fish. A
somewhat different approach is taken with aquatic invertebrates, some of which are more
sensitive to DDVP than fish by a factor of over 2500. Risks to sensitive species of aquatic
invertebrates – i.e., daphnids and other small arthropods – are characterized based on the lowest
reported LC_{50} value, 0.00007 mg/L from a 48-hour bioassay in *Daphnia pulex*. Some other
groups of aquatic invertebrates, such as snails, appear to be much less sensitive than small
arthropods. Risks to such tolerant species are based on a LC_{50} value of 21 mg/L in a freshwater
snail.

4.3.2. Toxicity to Terrestrial Organisms
Two different types of exposure assessments are given for terrestrial vertebrates: direct
consumption of all or part of the DDVP-PVC stip (Section 4.2.2.1) and consumption of water
contaminated with DDVP (4.2.2.2). The former scenario involves exposure to the formulated
DDVP and the latter exposure scenario involves exposure to unformulated or free DDVP. For
the exposure assessment involving direct consumption of the DDVP-PVC strip, the dose of 240
mg/kg for neurotoxicity from the study by Stanton et al. (1979) will be used to characterize risk.
No mortality or frank signs of AChE inhibition were observed at this dose. For exposure to free
DDVP in water, the NOAEL of 0.5 mg/kg for changes in AChE activity and other signs of
neurotoxicity will be used to characterize risk. This is the NOAEL selected by the U.S. EPA
(2000a) as the basis for the acute oral RfD for DDVP. As indicated in Section 4.4., these two
NOAEL values are substantially below the corresponding exposure levels. Thus, elaboration of
the dose-response assessment is not necessary.

4.3.3. Aquatic Organisms
4.3.3.1. Fish – The U.S. EPA typically uses LC_{50} values as benchmark doses for developing
acute hazard quotients and the most sensitive LC_{50} of 0.183 mg/L was used by U.S. EPA in it's
ecological risk assessment for DDVP (U.S. EPA 1999a, p. 29). USDA risk assessments typically
prefer to use NOEC (no observed effect concentrations) when such data are available. As
discussed in Section 4.1.3.1, WHO (1989) has identified an NOEC of 0.03 mg/L from studies
involving exposure periods of about 30 days. This NOEC will be adopted in the current risk
assessment. While the application of a 30-day NOEC to the acute and much shorter term

exposures considered in this risk assessment is likely to be over-protective, this has no impact on the characterization of risk because the anticipated levels of acute exposure are substantially below this NOEC. Also because this conservative NOEC value is below a level of concern, separate assessments are not made for sensitive and tolerant species of fish. This is discussed further in Section 4.4.

4.3.3.2. Aquatic Invertebrates – As noted in Section 4.1.3.3, some aquatic invertebrates are much more sensitive to DDVP than fish. Based on the lowest reported LC_{50} values in fish and invertebrates, some aquatic invertebrates are more sensitive than fish by a factor of over 2500. There is, however, a very wide range of tolerances in aquatic invertebrates. The lowest reported LC_{50} value is 0.00007 mg/L. This is a 48-hour LC_{50} value in *Daphnia pulex* reported by U.S. EPA (1999a, p. 13). A NOEC value is not reported by U.S. EPA (1999a). Thus, the LC_{50} 0.00007 mg/L is used directly in the risk characterization for sensitive aquatic invertebrates. As also noted in Section 4.1.3.3, the sensitivity of aquatic invertebrates to DDVP is highly variable. The least sensitive group of species appears to be aquatic snails, with a reported 96-hour LC_{50} of 21 mg/L (Jonnalagadda and Rao 1996). This value will be used to characterize risks in tolerant aquatic invertebrates.

4.4. RISK CHARACTERIZATION
4.4.1. Overview
As with the human health risk assessment, it is anticipated that typical exposures and consequent risks to nontarget species should be negligible. As with the human health risk assessment, it is anticipated that typical exposures and consequent risks to most nontarget species should be negligible. The containment of the DDVP within a slow release PVC strip combined with the target specific nature of pheromone baited traps should reduce the risks of inadvertent effects in non-target species. Other insects and arthropods that may inadvertently enter the trap will probably be killed by DDVP vapor. While such inadvertent contact may occur, it is not likely to impact substantial numbers of nontarget insects or arthropods.

Because of the limited use of DDVP, a relatively small number of exposure scenarios – all of which might be considered accidental or incidental – are developed. For terrestrial mammals, contact with the pest strip could occur by an animal directly tampering with a trap or by an animal consuming water that had been accidentally contaminated with a DDVP strip. Adverse effects would not be expected in either case. In the case of accidental contamination of a small body of water with a DDVP strip, concentrations of DDVP in the water would be below the level of concern for fish by factors of about 50 to 500. Some aquatic invertebrates, however, might be affected. For the most sensitive species of aquatic invertebrates – i.e., small aquatic arthropods such as daphnids – exposures could substantially exceed laboratory LC_{50} values by factors of up to about 8. Exposures to tolerant aquatic invertebrates – such as snails – would be below a level of concern by a substantial margin – i.e., factors of about 30,000 to 300,000. The exposure assessments that serve as the bases for these risk characterizations are highly dependent on specific conditions – i.e., how much DDVP was in the strip at the time that the contamination occurred and the size of the body of water that was contaminated.

4.4.2. Terrestrial Organisms
There is no indication that adverse effects in terrestrial vertebrates are likely. This assessment is based on the exposure scenarios for a relatively small mammal – i.e., a raccoon – consuming all or part of a DDVP-PVC strip as well as a very small mammal consuming water that had been contaminated with a pest strip.

The former scenario, direct consumption, may be plausible but is clearly extreme. The upper range of the exposure assessment assumes that the animal consumes the entire strip with a resulting dose of about 100 mg/kg (Section 4.2.2.1). The assessment of risk is based on a controlled laboratory study using a DDVP-PVC formulation in which no mortality was observed at 1,000 mg/kg and no signs of AChE inhibition were apparent at 240 mg/kg (Section 4.3.2). The dose of 100 mg/kg associated with upper range of the hazard quotient, 0.4, is below the the NOAEL by a factor 2.5.

The scenario for the consumption of contaminated water is based the assumption that a fresh DDVP strip inadvertently contaminates a small pond and, at the upper range of the estimated dose, the further assumption that all of the DDVP in the strip leaches into the water (Section

4.2.2.2 and Worksheets D02). The estimated dose is probably higher and perhaps much higher than what might actually occur because degradation of the DDVP in water is not considered. Even with these highly protective assumptions, the upper range of the risk quotient is only 0.0002 – i.e., below the level of concern (1) by a factor of 5,000. Thus, there is no plausible basis for asserting that adverse effects are likely.

No quantitative risk characterization is presented for terrestrial invertebrates. This approach is taken because there is no reason to anticipate that significant exposures to nontarget invertebrates are likely. It is possible that some insects and perhaps other arthropods could inadvertently enter a milk carton trap. In such a case, it is likely that the nontarget organisms would be killed by the DDVP vapor. While this is the intended effect in the target species, the gypsy moth, the efficacy of the traps is dependant on the use of another agent, disparlure, that serves as an attractant to male gypsy moths. As discussed in the risk assessment for disparlure, this attractant is highly specific to the gypsy moth and will not attract other species. Thus, the numbers of nontarget species that might be killed by inadvertently entering the traps is likely to be small and inconsequential.

4.4.3. Aquatic Organisms
4.4.3.1. Fish – There is no indication that fish are likely to be adversely affected by the use of DDVP in PVC strips. The exposure assessment for fish (Section 4.2.4) is based on the same very conservative exposure assessment used for mammals – i.e., the concentrations in water are likely to be over-estimated. The dose-response assessment is based on a 30-day NOEC for sublethal effects. The resulting risk quotients – i.e., 0.002 to 0.2 – are below the level of concern by factors of 50 to 500.

4.4.3.2. Aquatic Invertebrates – As discussed in Section 4.3.3.2, some aquatic invertebrates are much more sensitive to DDVP than fish and this difference in sensitivity impacts the characterization of risk. Based on the same conservative exposure assessment used for both fish and terrestrial vertebrates, some sensitive aquatic invertebrates could be adversely affected by DDVP contamination of water. As in the other exposure assessments involving contaminated water, this exposure scenario should be regarded as accidental rather than routine. In other words, under normal circumstances, water contamination from DDVP strips will be negligible and this is consistent with the conclusions reached by U.S. EPA (1999a, p. 25). Nonetheless, based on the modeled concentrations in the event of the accidental deposition of a strip containing 590 mg of DDVP into a small pond, concentrations of DDVP in the water would reach or substantially exceed the LC_{50} value for sensitive invertebrates and substantial mortality in sensitive invertebrates could occur.

The actual extent of mortality would depend on the rate at which DDVP is released from the strip, the degree of mixing that occurs in the water, and the rate of breakdown and dissipation of DDVP. These processes cannot be generically modeled but the conservative exposure assessment used to estimate concentrations in water suggests that adverse effects in sensitive aquatic invertebrates are plausible. No effects are likely in less sensitive aquatic invertebrates

such as aquatic snails. As discussed in Section 3.2.3.4, the hydrolysis of DDVP in water is rapid and it is likely that the estimates of adverse effects in some aquatic invertebrates would apply to only a very limited area near the pest strip rather than to the larger area of the body of water that is contaminated.

5. REFERENCES

Abdelsalam EB. 1999. Neurotoxic potential of six organophosphorus compounds in adult hens. Vet Hum Toxicol. 41(5):290-292.

ACGIH (American Conference of Governmental Industrial Hygienists). 1991. Documentation of the Threshold Limit Values and Biological Exposure Indices. 6th ed. Dichlorvos. Cincinnati, OH: American Conference of Governmental Industrial Hygienists; 446-448.

ACGIH (American Conference of Governmental Industrial Hygienists). 2004. TLV/BEI Development Process: An Overview. http://www.acgih.org/TLV/DevProcess.htm.

Aratake Y; Kayamura T. 1973. [Toxicity of insecticides to silkworm larvae.] Sanshi Kenkyu (Acta serol.). 87: 68-78 (in Japanese). (As cited by WHO 1989).

Arimatsu S; Hoshiro Y; Nomura T. 1977. [Studies on primary irritation test of pesticides in rabbits.] Nippon Noson Igakkai Zasshi. 26572-573. (In Japanese) As cited by WHO 1989.

ARS (USDA/Agricultural Research Station). 1995. ARS Pesticide Properties Database. http://www.arsusda.gov/ppdb.html. Last updated May 1995

Atkins EL; Greywood EA; Macdonald RL. 1973. Toxicity of pesticides and other agricultural chemicals to honey bees. University of California (Agricultural Extension Report No. M-16 Rev. 9/73). (As cited by WHO 1989).

ATSDR (Agency for Toxic Substances and Disease Registry). 1993. Case Studies in Environmental Medicine No. 22: Cholinesterase Inhibiting Pesticide Toxicity. U.S. Department of Health and Human Services, Public Health Service. September, 1993. Available at: http://books.nap.edu/books/0309051401/html/584.html.

ATSDR (Agency for Toxic Substances and Disease Registry). 1995. Toxicological Profile for Xylene. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Division of Toxicology, Toxicology Information Branch, Atlanta, Ga. August, 1995. Available at: http://www.atsdr.cdc.gov/toxprofiles/

ATSDR (Agency for Toxic Substances and Disease Registry). 1997. Toxicological Profile for Dichlorvos. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Division of Toxicology, Toxicology Information Branch, Atlanta, Ga. Sep. 1997. Available at: http://www.atsdr.cdc.gov/toxprofiles/

Bast CB; Milanez S; Forsyth CS. 1997. Data Evaluation Report: Dichlorvos, Study Type, Subchronic Oral Neurotoxicy. MRID No. 42958101, 42655301, 41593101, 41951501, and 40299401, CHEG-000155.

Bisby JA; Simpson GR. 1975. An unusual presentation of systemic organophosphate poisoning. Med J Aust. 2:394-395.

Blair D; Hoadley EC; Hutson DH. 1975. The distribution of dichlorvos in the tissues of mammals after its inhalation or intravenous administration. Toxicol Appl Pharmacol. 31(2):243-253.

Blair D; Dix KM; Hunt PF; Thorpe E; Stevenson DE; Walker AIR. 1976. Dichlorvos – a 2-year inhalation carcinogenesis study in rats. Arch Toxicol (Berlin). 35:281-294.

Braun WG; Killeen JC Jr. 1975. Acute Oral Toxicity in Rats: Compound No. L-65-39: Project No. 2564-75. MRID 00029130.

Brown VKH; Blair D; Holmes DL; Pickering RG. 1968. The toxicity of low concentrations of dichlorvos by inhalation in rodent and avian species. Sittingbourne, Shell Research Ltd (Unpublished Report No. TLGR.0015.68). (As cited by WHO 1989).

Bryant D.H. 1985. Asthma due to insecticide sensitivity. Aust NZ J Med. 15:66-68. As cited by Vial et al. 1996.

Budavari S. (Ed). 1989. The Merck Index: An Encyclopedia of Chemicals, Drugs,and Biologicals, 11th ed. Merck & Co., Inc., Rahway, New Jersey.

Butler GL. 1977. Algae and pesticides. Residue Rev. 66: 40. (As cited by WHO 1989).

Calabrese EJ. 1991. Multiple chemical interactions. Chelsea, Michigan: Lewis Publishers; 355-385.

Casale GP; Cohen SD; DiCapua RA. 1983. The effects of organophosphate-induced cholinergic stimulation on the antibody response to sheep erythrocytes in inbred mice. Toxicol Appl Pharmacol. 68:198-205.

Cavegna G; Locati G; Vigliani EC. 1969. Clinical effects of exposure to DDVP (Vapona) insecticide in hospital wards. Arch Environ Health. 19:112-123.

Cervoni WA; Oliver-Gonzales J; Kaye S; Slomka MB. 1969. Dichlorvos as a single-dose intestinal anthelmintic therapy for man. Am J Trop Med Hyg 18:912.

Charles KE; Veitch JA. 2002. Outdoor Ventilation Rates in Offices and Occupant Satisfaction. National Research Council Canada, Institute for Research in Construction. Report IRC-RR-160. Available at: http://irc.nrc-cnrc.gc.ca/ircpubs.

Civen M; Leep JE; Wishnow RM; Wolfsen A; Morin RJ. 1980. Effects of low level administration of dichlorvos on adrenocorticotrophic hormone secretion, adrenal cholesteryl ester and steroid metabolism. Biochem Pharmacol (Oxford). 29:635-641.

Clements RG; Nabholz JV; Zeeman M. 1996. Estimating Toxicity of Industrial Chemicals to Aquatic Organisms Using Structure-activity Relationships. Available at: http://www.epa.gov/oppt/newchems/sarman.pdf .

Clinch PG. 1970. Effect on honey bees of combs exposed to vapour from dichlorvos slow-release strips. NZ J Agric Res. 13(2): 448-452.

Collins RD; DeVries DM. 1973. Air concentration and food residues from use of Shell's No-Pest Insecticide Strip. Bull Environ Toxicol. 9(4):227-233.

Collins JA; Schooley MA; Singh VK. 1977. The effect of dietary dichlorvos on swine reproduction and viability of their offspring. Toxicol Appl Pharm. 19:377.

Colosio C; Corsini E; Barcellini W; Maroni M. 1999. Immune parameters in biological monitoring of pesticide exposure: Current knowledge and perspectives. Toxicol Lett (Shannon). 108(2-3):285-295.

Costa LG; Murphy SD. 1984. Interaction between acetaminophen and organophosphates in mice. Chem Pathol Pharmacol. 44(3):389-400.

Cunningham ML; Matthews HB. 1995. Cell proliferation as a determining factor for the carcinogenicity of chemicals: Studies with mutagenic carcinogens and mutagenic noncarcinogens. Toxicol Lett. 82-83:9-14.

Dambska M; Iwanowski L; Kozlowski P. 1979. The effect of transplacental intoxication with dichlorvos on the development of cerebral cortex in newborn rabbits. Neuropatologia Polska (Warszawa) 17:571-576.

Das YT; Taskar PK; Brown HD; Chattopadhyay SK. 1983. Exposure of professional pest control operator to dichlorvos (DDVP) on house structures. Tox Letters. 17:95-99.

Davis, J.R.; Brownson, R.C.; Garcia, R. 1992. Family pesticide use in the home, garden orchard, and yard. Arch Environ Contam Toxicol. 22:260-266.

Davis, J.F.; Brownson, R.C.; Garcia, R.; Bentz, B; Turner, A. 1993. Family pesticide use and childhood brain cancer. Arch Environ Contam Toxicol. 24:87-92.

Dean BJ, Thorpe E. 1972. Studies with dichlorvos vapor in dominant lethal mutation tests on mice. Arch Toxicol. 30(1):5l-59.

Dellinger JA; McKiernan BC; Koritz GD; Richardson BC. 1987. Latent dichlorvos neurotoxicity detected by vagal tone monitoring in dogs. Neurotoxicol Teratol. 9:197-201.

Desi I; Varga L; Farkas I. 1978. Studies on the immunosuppressive effect of organochlorine and organophosphoric pesticides in subacute experiments. J Hyg Epidemiol Microbiol Immunol (Praha). 22:115-122.

Desi I; Varga L; Farkas I. 1980. The effect of DDVP, an organophosphorus pesticide, on the humoral and cell-mediated immunity of rabbits. Arch Toxicol Supp (Berlin). 4:171-174.

Desi I; Nagymajtenyi L; Schulz H; Nehez M. 1998. Epidemiological investigations and experimental model studies on exposure of pesticides. Toxicol Lett (Shannon). 96-97(Spec. Issue):351-359.

Dunier M; Siwicki AK; Demael A. 1991. Effects of organophosphorus insecticides: Effects of trichlorfon and dichlorvos on the immune response of carp (*Cyprinus carpio*), III. *In vitro* effects on lymphocyte proliferation and phagocytosis and *in vivo* effects on humoral response. Ecotoxicol Environ Saf. 22:79-87.

Durham WF; Gaines TB; McCauley RH; Sedlak VA; Mattson AM; Hayes WJ. 1957. Studies on the toxicity of O,O,-Dimethyl-2,2-dichlorovinyl phosphate (DDVP). AMA Arch Indus Health. 15:340- 349.

Durkin PR; Diamond G. 2002. Neurotoxicity, Immunotoxicity, and Endocrine Disruption with Specific Commentary on Glyphosate, Triclopyr, and Hexazinone: Final Report. SERA TR 01-43-08-04a dated January 30, 2002. Available at www.fs.fed.us/foresthealth/pesticide/risk.htm.

Ecobichon DJ. 2001. Toxic Effects of Pesticides. In: Klaassen, C.D., ed. Casarett and Doull's Toxicology: The Basic Science of Poisons, Sixth Edition. New York: McGraw-Hill; 774-784.

Ehrich M; Cohen SD. 1977. DDVP (Dichlorvos) detoxification by binding and interactions with DDT, dieldrin, and malaoxon. J Toxicol Environ Health. 3:491-500.

Eisler R. 1970.   Acute toxicities of organochlorine and organophosphorus insecticides to estuarine fish. Washington DC, US Department of the Interior, Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife (Technical Paper No. 46). (As cited by WHO 1989).

England DC; Knight AD; Day PE; Kennick WH; Oldfield JE. 1969. Influence of dichlorvos on blood sugar, ovulation rate, and early embryo mortality in gilts. Proc West Sec Amer Soc Anim Sci. 20:73-78.

 Fedoruk MJ; Kerger BD. 2003. Measurement of volatile organic compounds inside automobiles. Journal of Exposure Analysis and Environmental Epidemiology. 13: 31- 41.

Fernandes MD; Queiroz M LS. 1999. Measurement of the respiratory burst and chemotaxis in polymorphonuclear leukocytes from Anti-ChE insecticides-exposed workers. Immunopharmacol Immunotoxicol. 21(3):621-633.

Fernandez G; Diaz Gomez MI; Castro JA. 1975. Cholinesterase inhibition by phenothiazine and nonphenothiazine antihistaminics: Analysis of its postulated role in synergizing organophosphate toxicity. Tox Appl Pharmacol. 31:179-190.

Forget J; Pavillon JF; Menasria MR; Bocquene G. 1998. Mortality and LC_{50} values for several stages of the marine copepod *Tigriopus brevicornis* (Muller) exposed to the metals arsenic and cadmium and the pesticides atrazine, carbofuran, dichlorvos, and malathion. Ecotoxicol Environ Saf. 40(3):239-244.

Fujita K; Matsushima S; Abe E; Sasaki K; Kurosawa, K. 1977. [Examination of the effects of dichlorvos on the testis.] Nippon Noson Igakkai Zasshi. 26(3)328-329. (In Japanese)

Fukami J. 1980. Metabolism of several insecticides by glutathione-S-transferases. Pharmacol Ther. 10:437-514. As cited by Costa and Murphy 1984.

Gage JC. 1967. The significance of blood cholinesterase activity measurements. Residue Reviews. 18:159-173.

Gaines TB. 1969. Acute toxicity of pesticides. Toxicol Appl Pharmacol. 14:513-534.

Gallo MA; Lawryk NJ. 1991. Organic phoshporus pesticides. In: Hayes, W.J.; Laws, E.R., eds. Handbook of pesticide toxicology, Volume 2, Classes of pesticides. San Diego: Academic Press, Inc.; 917-1123.

Garcia-Repetto R; Martinez D; Repetto M. 1995. Malathion and dichlorvos toxicokinetics after the oral administration of malathion and trichlorfon. Vet Human Toxicol. 37(4):306-309.

Gillett JW; Harr JR; Lindstrom FT; Mount DA; St. Clair AD; Weber LJ. 1972a. Evaluation of human health hazards on use of dichlorvos (DDVP), especially in resin strips. Residue Reviews 44:115-159.

Gillett JW; Harr JR; St. Clair AD; Weber LJ. 1972b. Comment on the distinction between hazard and safety in evaluation of human health hazards on use of dichlorvos, especially in resin strips. Residue Reviews 44:161-184.

Gledhill A. 1997. Dichlorvos: A study to investigate the effect of a single oral dose on erythrocyte cholinesterase inhibition in healthy male volunteers: Lab Project Number: CTL/P/5393: XH6064. Unpublished study prepared by Zeneca Central Toxicology Lab. 44 p. MRID No. 44248802. Available from U.S. EPA/OPP/CBI Office.

Gold RE; Holcslaw T; Tupy D; Ballard JB. 1984. Dermal and respiratory exposure to applicators and occupants of residences treated with dichlorvos (DDVP). J Econ Entomol. 77(2):430-436.

Goldstein A; Aronow L; Kaman SM. 1974. Principles of Drug Action: The Basis of Pharmacology. 2nd ed. John Wiley and Sons, New York, NY. 854 p.

Grahl K; Horn H; Hallebach R. 1981. [Effect of butonate, trichlorfon, and dichlorvos on plankton populations.] Acta hydrochim. Hydrobiol., 9(2): 147-161 (in German). (As cited by WHO 1989).

Hass DK; Collins JA; Kodamma JK. 1971. Effects of orally administered dichlorvos in rhesus monkeys. J Amer Vet Med Assoc. 161(6):714-719.

Hattori K; Sato H; Tsuchiya K; Yamamoto N; Ogawa E. 1974. [Toxicological studies on the influences of chemicals to the birds. I. Oral acute toxicity and cholinesterase inhibition of three organophosphate insecticides in Japanese quail.] Hokkaidoritsu Eisei Kenkyusho Ho. 24: 35-38 (in Japanese, with English summary). (As cited by WHO 1989).

Health-Chem Corporation. 19??. Hercon Vaportape II Insecticidal Strips for Use as Toxicant in Insect Traps. MRID 00084822.

Hercon (Hercon Environmental Company). 1978. Application for pesticide registration: Hercon Disparlure dispenser. Located at: U.S. Department of Agriculture, Forest Service, Radnor, PA.

Hercon (Hercon Environmental Company) [Label for Vaportape II and memo to Noel Schneeberger]. 1993 April 20. Located at: U.S. Department of Agriculture, Forest Service, Radnor, PA.

Hercon (Hercon Environmental Company) [Facsimilie to Patrick Durkin]. 1994 April 13. Hercon Vaportape II: Release rate study, Rep 1 - Lot No. 0061V; Study date: October 9, 1991. Located at: U.S. Department of Agriculture, Forest Service, Radnor, PA.

Hercon (Hercon Environmental Company). 2004. VaporTape II label. Downloaded on May 29, 2004 from: http://www.herconenviron.com/pdf/hercon_vaportape.pdf

Hercon Products Group. 19??. DDVP Toxicity to Mammals. (Unpublished study received Oct 16, 1981 under 8730-32; submitted by Herculite Products, Inc., New York, N.Y.; CDL:246081-D) MRID 00084824.

Herculite Products Incorporated. 19??a. Hercon Vaportape II Insecticidal Strips. MRID 00084825.

Herculite Products Incorporated. 19??b. Study of the Chemical Hercon Vaportape. Includes undated method entitled: DDVP analysis in Hercon dispensers. MRID 00084823.

Hill EF; Heath RG; Spann JW; Williams JD. 1975. Lethal dietary toxicities of environmental pollutants to birds. Washington DC, US Department of the Interior, Fish and Wildlife Service, pp. 1-51 (Special Scientific Report: Wildlife No. 191). (As cited by WHO 1989).

Hine CH; Slomka MB. 1968. Human tolerance of the acute and subacute oral administration of a polyvinylchloride formulation of dichlorvos (V-3 and V-12). Pharmacologist. 10222. Cited by WHO 1989.

Hine CH; Slomka MB. 1970. Human toxicity studies on polyvinyl chloride formulation of dichlorvos. Toxicol Appl Pharmacol. 17:304.

Hour TC; Chen L; Lin JK. 1998. Comparative investigation on the mutagenicities of organophosphate, phthalimide, pyrethroid and carbamate insecticides by the ames and lactam tests. Mutagenesis. 13(2):157-66.

IARC (International Agency for Research on Cancer). 1979. Dichlorvos. In: IARC monographs on the evaluation of carcinogenic risks to humans: Some halogenated hydrocarbons. Volume 20; 97-127.

IARC (International Agency for Research on Cancer). 1991. Dichlorvos. In: IARC monographs on the evaluation of carcinogenic risks to humans: Occupational exposures in insecticide application, and some pesticides. 53:267-307.

ICRP (International Commission on Radiologic Protection). 1975. Report of the Task Group on Reference Man. Recommendations of the International Commission on Radiological Protection (ICRP) Publ. No. 23. Pergamon Press, New York, NY.

Institäoris L; Siroki O; Fekete K; Däesi I. 1995. Immunotoxicological investigation of repeated small doses of dichlorvos (DDVP) in three generations of rats. Int J Environ Health Res. 5(3):239-45.

Institoris L; Nagymajtenyi L; Siroki O; Desi I. 1997. Comparison of the immuno- and neurotoxicological effects of repeated small doses of an organophosphate pesticide, ddvp, in three generations of rats. Neurotoxicol. 18(3):898.

Jacobs DE. 1968. Experiences with a broad-spectrum anthelmintic, Dichlorvos, in the adult pig. Vet Rec. 83:160-164.

Jakubowska B; Nowak A. 1973. [The effect of organophosphorus and carbamate insecticides on the development of soil fungi.] Zesz. Nauk. Akad. Roln. Szczecinie, 39(10): 141-150 (in Polish). (As cited by WHO 1989).

Jeffcoat A. 1990. Dermal absorption of dichlorvos in rats: Lab Project Number: 4615. Unpublished study prepared by Research Triangle Institute. 196 p. MRID No. 41435201.

Jian T; Zhiying F. 1990. Chronotoxicologic studies on dichlorphos in mice and humans. Chronobiology: Its Role in Clinical Medicine, General Biology, and Agriculture. Part A. pp. 503-510.

Johnson, M.K. 1978. The anomalous behaviour of dimethyl phosphates in the biochemical test for delayed neurotoxicity. Arch Toxicol. 41:107-110.

Johnson MK. 1981. Delayed neurotoxicity: Do trichlorphon and/or dichlorvos cause delayed neuropathy in man or in test animals? Acta Pharmacol Toxicol (Copenhagen). 49(Suppl. 5):87-98.

Johnson WW; Finley MT. 1980. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates, Washington DC, US Department of the Interior, Fish and Wildlife Service (Resource Publication No. 137). (As cited by WHO 1989).

Jokanovic M; Kosanovic M; Maksimovic M. 1996. Interaction of organophosphorus compounds with carboxylesterases in the rat. Arch Toxicol. 70(7):444-450.

Jones KH; Sanderson DM; Noakes DN. 1968. Acute toxicity data for pesticides. World Review of Pest Control. l 7:135-143.

Jonnalagadda PR; Rao BP. 1996. Histopathological changes induced by specific pesticides on some tissues of the fresh water snail *Bellamya dissimilis*. Bull Environ Contam Toxicol. 57(4):648-654.

Julka D; Pal R; Gill KD. 1992. Neurotoxicity of dichlorvoseffect on antioxidant defense system in the rat central nervous system. Exp Mol Patho1. 56(2):144-152.

Khera KS; Lyon DA. 1968. Chick and duck embryos in the evaluation of pesticide toxicity. Toxicol Appl Pharmacol. 13:1-15.

Kimbrough RD; Gaines TB. 1969. Effect of organic phosphorus compounds and alkylating agents on the rat fetus. Arch Environ Health. 16: 805-808.

 Kodama JK. 1960. Technical DDVP. Acute oral administration dogs, Vienna, Virginia, Hazleton Laboratories (Unpublished Report, 20 January). As cited by WHO 1989.

Korninger HC; Lenz K. 1978. Poisoning in childhood – an information center report. Wiener Klinische Wochenschrift (Wien). 90:1-7.

Lal R. 1982. Accumulation, metabolism, and effects of organophosphorus insecticides on microorganisms. Adv. Appl. Microbiol. 28: 149-200. (As cited by WHO 1989).

Lamoreaux RJ; Newland LW. 1978. The fate of dichlorvos in soil. Chemosphere. 10:807-814.

Laws ER. 1966. Route of absorption of DDVP after oral administration to rats. Toxicol Appl Pharmacol. 8:193-196.

Leary JS; Keane WT; Fontenot C; Feichtmeir E; Schultz D; Koos B; Hirsch L; Lavor EM; Roan CR; Hine CH. 1974. Safety evaluation in the home of polyvinyl chloride resin strip containing dichlorvos (DDVP). Arch Environ Health. 29:308-314.

Leonard D. 2004. Review comments on SERA TR 04-43-05-05a, Control/Eradication Agents for the Gypsy Moth – Human Health and Ecological Risk Assessment for DDVP (Dichlorvos), Peer Review Draft dated July 8, 2004. Comments by Donna Leonard, USDA Forest Service, Forest Health Protection, Asheville, NC. Comments received via email from dleonard@fs.fed.us.

Lieberman MT; Alexander M. 1981. Effects of pesticides on decomposition of organic matter nitrification in sewage.   Bull Environ Contam Toxicol. 26: 554-560.

Liess; Savitz. 1995. Home pesticide use and childhood cancer: A case-control study. Am J Public Health. 85:249-252.

Lloyd JE; Matthysse JG. 1971. Residues of dichlorvos, diazinon, and dimetilan in milk of cows fed PVC-insecticide feed additives. J Econ Entomol. 64(4):821-822.

 Liebhold AM; McManus M. 1999. The evolving use of insecticides in gypsy moth management. J For. 97(3): 20-23.

Lopez JD. 1998. Evaluation of various operational aspects for sex pheromone trapping of beet armyworm. Southwest Entomol. 23(4):301-307.

Maddy KT; Goh KS; Meinders DD; Edmiston S; Margetich S. 1984. Dissipation of dislodgeable residue of chlorpyrifos and DDVP on turf. California Department of Food and Agriculture, Division of Pest Management, Environmental Protection and Worker Safety, Worker Safety and Health Unit, Sacramento, CA. As cited by USDA 1995b.

Manley A, Brown WR, Mennear J. 1997. Dichlorvos and mononuclear cell leukemia in Fischer 344 rats: Lack of effect of chronic administration on progression of the disease. Int J Toxicol. 16(1):1-7.

Mason HJ. 2000. The recovery of plasma cholinesterase and erythrocyte acetylcholinesterase activity in workers after over-exposure to dichlorvos. Occup Med (Lond). 50(5): 343-7.

Mathias CGT. 1983. Persistent contact dermatitis from the insecticide dichlorvos. Contact Dermatitis. 9:217-218.

Mennear JH. 1994. Dichlorvos carcinogenicity: An assessment of the weight of experimental evidence. Regul. Toxicol. Pharmacol. 20354-361. As cited by Mennear 1998.

Mennear JH. 1998. Dichlorvos a regulatory conundrum. Regul Toxicol Pharmacol. 27(3):265-272.

Meylan W; Howard P. 2000. DDVP Output from EPI-SUITE – Estimation Program Interface, Version 3.11. Syracuse Research Corporation, Syracuse, N.Y. For: U.S. Environmental Protection Agency, Office of Pollution prevention and Toxics, Washington, D.C.

Michalek H; Stavinoha WB. 1978. Effect of chlorpromazine pre-treatment on the inhibition of total cholinesterases and butyryl-cholinesterase in brain of rats poisoned by physostigmine or dichlorvos. Toxicology. 9:205-218.

Mohammad FK; St. Omer VE. 1983. Interaction of dichlorvos-crotoxyphos insecticide with phenothiazine anthelmintic in sheep with or without *Haemonchus* and *Trichostrongylus* infections. Am J Vet Res. 44:1949-1953.

Mohammad FK; St Omer VE. 1985. Toxicity and interaction of topical organophosphate insecticide dichlorvos crotoxyphos and phenothiazine anthelmintic in sheep previously exposed to both drugs. Vet Hum Toxicol. 27:181-184.

Moriya M; Ohta T; Watanabe K; Miyazawa T; Kato K; Shirasu Y. 1983. Further mutagenicity studies on pesticides in bacterial reversion assay systems. Mut Res. 116:185-206.

Muller GH. 1970. Flea collar dermatitis in animals. J Am Vet Med Assoc. 157(11):1616-1626.

Murphy SD. 1980. Assessment of the potential for toxic interactions among environmental pollutants. In: The Principles and Methods in Modern Toxicology. Galli CL; Murphy SD; Paoletti R (eds.). pp. 277-294.

Naidu NV; Reddy KS; Janardhan A; Murthy MK. 1978. Toxicological investigation of dichlorvos in chicks. Indian J. Pharmacol. 10(14): 323-326. (As cited by WHO 1989).

NRC (National Research Council). 1983. Risk assessment in the Federal government: managing the process. Washington, DC: National Academy Press; 176 p. + app.

NCI (National Cancer Institute). 1977. Bioassay of Dichlorvos for Possible Carcinogenicity. Washington, DC: U.S. Department of Health Education and Welfare. Available from NTIS, Springfield, VA: PB 270 937.

NIOSH (National Institute for Occupational Safety and Health). 2002. Pocket Guide to Guide to Chemical Hazards: Dichlorvos. U.S. Department of Health and Human Services. Available at: http://www.cdc.gov/niosh/npg/npg.html.

NTP. 1989. Toxicology and Carcinogenesis Studies of Dichlorvos (CAS No. 62-73-7) in F344/N rats and B6C3F1 Mice (Gavage Studies). National Toxicology Program. Washington, DC: U.S. Department of Health and Human Services. Technical Report Series No. 342.

Omkar; Shukla GS. 1984. Alteration in carbohydrate metabolism of fresh-water prawn *Macrobianchium lamarrei* after dichlorvos exposure. Ind. Health. 22: 133-136. (As cited by WHO 1989).

Pena-Chavarria A; Swartzwelder JC; Villarejos VM; Kotcher E; Arguedas J. 1969. Dichlorvos, an effective broad-spectrum anthelmintic. Am J Trop Med Hyg. 18:907.

Potter JC; Boyer AC; Marxmiller RL; Young R; Loeffler JE. 1973. Radioisotope residues and residues of dichlorvos and its metabolites in pregnant sows and their progeny dosed with dichlorvos-^{14}C or dichlorvos-^{36}Cl formulated as PVC pellets. J Agric Food Chem. 21(4):734-738.

Purshottam T; Kaveeshwar U. 1982. Effect of phenobarbital pretreatment on regeneration of plasma cholinesterase activity inhibited by parathion or dichlorvos. Arch Environ Health. 37(1):53-58.

Qiao CL; Sun ZQ; Liu JE. 1999. New esterase enzymes involved in organophosphate resistance in *Culex pipiens* (Diptera: Culicidae) from Guang Zhou, China. J Med Entomol. 36(6):666-670.

Queiroz MLS; Fernandes MD; Valadares MC. 1999. Neutrophil function in workers exposed to organophosphate and carbamate insecticides. Int J Immunopharmacol. 21(4):263-270.

Radeleff RD; Woodard GT. 1957. The toxicity of organic phosphorus insecticides to livestock. J. Am. Vet. Med. Assoc. 130: 215-216. (As cited by WHO 1989).

Ramel C; Drake J; Sugimura T. 1980. An evaluation of the genetic toxicity of Dichlorvos. Mut Res (Amsterdam). 76:297-309.

Rath S; Misra BN. 1979. Sub-lethal effects of dichlorvos (DDVP) on respiratory metabolism of *Tilapia mossambica* of 3 age groups. Exp. Gerontol. 14: 37-41. (As cited by WHO 1989).

Rath S; Misra BN. 1980. Pigment disperson in *Tilapia mossambica* Peters exposed to dichlorvos (DDVP). Curr. Sci. 49(23): 907-909. (As cited by WHO 1989).

Rath S; Misra BN. 1981. Toxicological effects of dichlorvos (DDVP) on brain and liver acetylcholinesterase (AChE) activity of *Tilapia mossambica* Peters. Toxicology. 19: 239-245. (As cited by WHO 1989).

Reeves, J.D.; Driggers, D.A.; Kiley, V.A. 1981. Household insecticide associated aplastic anaemia and acute leukaemia in children. Lancet (London). August 8: 300-301.

Sakaguchi K; Nagayama M; Masaoka T; Nishimura A; Kageyama K; Shirai M; Akahori F. 1997. Effects of fenthion, isoxathion, dichlorvos and propaphos on the serum cholinesterase isoenzyme patterns of dogs. Vet Hum Toxicol. 39(1):1-5.

Sarin S; Gill KD. 1997. *In vitro* and *in vivo* interaction of dichlorvos with neuropathy target esterase in rat brain. FASEB Journal. 11(9):A1228.

Sarin S; Gill KD. 1998. Biochemical and behavioral deficits in adult rat following chronic dichlorvos exposure. Pharmacol Biochem Behav. 59(4):1081-1086.

Sarin S; Gill KD. 2000. Biochemical characterization of dichlorvos-induced delayed neurotoxicity in rat. IUBMB Life. 49(2):125-130.

Schafer EW. 1972. The acute oral toxicity of 369 pesticidal, pharmaceutical, and other chemicals to wild birds. Toxicol. Appl. Pharmacol. 21: 315-330. (As cited by WHO 1989).

Schafer EW; Brunton RB. 1979. Indicator bird species for toxicity determinations: Is the technique usable in test method development? In: Beck, J.R; ed. Vertebrate Pest Control and Management Materials. Philadelphia, Pennsylvania, American Society for Testing and Materials, pp. 157-168 (ASTM STP 680). (As cited by WHO 1989).

Schulz H; Nagymajtenyi L; Desi I. 1995. Life-time exposure to dichlorvos affects behaviour of mature rats. Hum Exp Toxicol. 14(9):721-726.

Schwetz BA; Ioset HD; Leong BKJ; Staples RE. 1979. Teratogenic potential of dichlorvos given by inhalation and gavage to mice and rabbits. Teratology. 20:383-388.

Schlinke JC; Palmer JS. 1973. Combined effects of phenothiazine and organophosphate insecticides in cattle. J Amer Vet Med Assoc. 163:756-758.

Schwetz, B.A.; Ioset, H.D.; Leong, B.K.J.; Staples, R.E. 1979. Teratogenic potential of dichlorvos given by inhalation and gavage to mice and rabbits. Teratology. 20:383-388.

SERA (Syracuse Environmental Research Associates, Inc.). 2001. Preparation of Environmental Documentation and Risk Assessments, SERA MD 2001-01a, draft dated July 2001. Syracuse Environmental Research Associates, Inc., Fayetteville, NY. Available at www.sera-inc.com.

SERA (Syracuse Environmental Research Associates, Inc.). 2004. Documentation for the Use EXCEL Worksheets in Forest Service Risk Assessments (Version 3.01), SERA TD 2004-03.01a, dated March 13, 2004. Available at: www.sera-inc.com.

Shell Chemical Company. 1972. Summary of Basic Data for Vapona Insecticide. Rev. San Ramon, Calif.: Shell. MRID 00049640.

Shimizu K; Shiono H; Fukushima T; Sasaki M; Akutsu H; Sakata M. 1996. Tissue distribution of DDVP after fatal ingestion. Forensic Sci Int. 83(1): 61-66.

Shimizu K; Sasaki M; Fukushima T; Shiono H. 1997. A fatal case of DDVP poisoning: Biochemical and toxicological findings. Res Pract Forensic Med. 40(0):183-187.

Shinkaji N; Adachi T. 1978. [The effect of certain pesticides on the predaceous mite *Amblyseius longispinosus* (Evans) (Acarina: Phytoseiidae).] Akitsu. 2: 99-108 (in Japanese, with English tables). (As cited by WHO 1989).

Short CE; Cuneio J; Cupp D. 1971. Organophosphate-induced complications during anesthetic management in the horse. J Amer Vet Med Assoc. 159(11):1319-1327.

Siers DG; DeKay DE; Mersmann HJ; Brown LJ. 1976. Late gestation feeding of dichlorvos: A physiological characterization of the neonate and a growth-survival response. J Anim Sci. 42(2):381-392.

Siers DG; Danielson DM; Chai EY; Keasling HH. 1977. Late gestation feeding of dichlorvos: The response in artificially and dam-reared litters. J Anim Sci. 44(1):1-7.

Singh VK; Perkins CT; Schooley MA. 1968. Effects of dichlorvos fed to gravid sows on performance of their offspring to weaning. Midwestern Section Abstracts. pp. 1779-1780.

Slomka MB. 1970. Facts about No-Pest DDVP strips. Shell Chemical Co.; 18 p. As cited by Gillett et al. 1972a.

Slomka MB; Hine CH. 1981. Clinical pharmacology of Dichlorvos. Acta Pharmacol Toxicol (Copenhagen). 49(Suppl. V):105-108.

Starner N. 1993. Stability Study of dichlorvos in Hercon Vaportape II. Unpublished study prepared by Hercon Environmental Co. 123 p. MRID 43109301.

Stanton HC; Albert JR; Mersman HJ. 1979. Studies on the pharmacology and safety of dichlorvos in pigs and pregnant sows. Am J Vet Res. 40:315-320.

Stewart TB; Hale OM; Marti OG. 1975. Efficacy of two dichlorvos formulations against larval and adult *Hyostrongylus rubidus* in swine. Am J Vet Res. 36(6):771-772.

Sturm A; Hansen PD. 1999. Altered cholinesterase and monooxygenase levels in *Daphnia magna* and *Chironomus riparius* exposed to environmental pollutants. Ecotoxicol Environ Saf. 42(1):9-15.

Taylor P. 1996. Anticholinesterase agents. In: Hardman, J.G. and Limbird, L.E., eds. Goodman and Gilman's The Pharmacological Basis of Therapeutics, Ninth Edition. New York: McGraw-Hill. 162-174.

Terayama K; Honma H; Kawarabayashi T. 1978. Toxicity of heavy metals and insecticides on slime mold *Physarum polycephalum*. J Toxicol Sci. 3: 293-304. (As cited by WHO 1989).

Thorpe E; Wilson AB; Dix KM; Blair D. 1972. Teratological studies with dichlorvos vapour in rabbits and rats. Arch Toxicol. 30:29-38.

Timmons EH; Chaklos RJ; Bannister TM; Kaplan HM. 1975. Dichlorvos effects on estrous cycle onset in the rat. Lab Anim Sci. 25(1):45-47.

Tracy RL; Woodcock, JG; Chodroff S. 1960. Toxicological Aspects of 2,2'-dichlorovinyl dimethylphosphate (DDVP) in Cows, Horses, and White Rats. J Econ Entomol. 53(4): 593-601. (As cited by WHO 1989).

Tucker RK; Crabtree DG. 1970. Handbook of toxicity of pesticides to wildlife. Washington DC, U.S. Department of the Interior, Fish and Wildlife Service, 43 pp (Resource Publication No. 84). (As cited by WHO 1989).

Tumbleson ME; Wescott RB. 1969. Serum biochemic values in piglets from sows fed dichlorvos prior to farrowing. J Cop Lab Med. 3:67-70.

Ueda A, Aoyama K, Manda F, Ueda T, Kawahara Y. 1994. Delayed-type allergenicity of triforine (saprol). Contact Dermatitis. 31 (3):140-145.

U.S. EPA (U. S. Environmental Protection Agency). 1975. Summary of reported episodes involving the impregnated resin strip, (No-Pest, Pest Strip) from January 1967 to March 1975. Pesticide Episode Review System, Report No. 35. Washington, DC: Pesticide Use Analysis Branch, Operations Division, Office of Pesticides Program; report dated April, 1975; 5 pp.

U.S. EPA (U. S. Environmental Protection Agency). 1981. Summary of reported pesticide incidents involving dichlorvos. Pesticide Incident Monitoring System, Report No. 403. Washington, DC: Health Effects Branch, Hazard Evaluation Division, Office of Pesticides Program; report dated January, 1981; 6 p.

U.S. EPA (U.S. Environmental Protection Agency). 1989. Recommendations for and Documentation of Biological Values for use in Risk Assessment. U.S. EPA, Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH. ECAO-CIN-554. [pagination not continuous].

U.S. EPA (U. S. Environmental Protection Agency). 1994. Integrated Risk Information System (IRIS). Washington, DC. Available online at: http://www.epa.gov/iris/.

U.S. EPA (U. S. Environmental Protection Agency). 1999a. Environmental Fate and Effects Division, Phase I Comments on Dichlorvos. Available at: http://www.epa.gov/pesticides/op/ddvp.htm.

U.S. EPA (U. S. Environmental Protection Agency). 1999b. Error in Resin Strip Exposure Assessment for Dichlorvos (DDVP), PC Code 084001, DP Code D257002. Memorandum from David Jaquith to Kimberly Lowe dated August 16, 1999. Available at: http://www.epa.gov/pesticides/op/ddvp/resin.pdf.

U.S. EPA (U. S. Environmental Protection Agency). 2000a. Revised Preliminary HED Risk Assessment for Dichlorvos. Available at: http://www.epa.gov/pesticides/op/ddvp.htm

U.S. EPA (U. S. Environmental Protection Agency). 2000b. Dichlorvos, Cancer Assessment Review Committee Final Report. Dated February 2, 2000. Available at: http://www.epa.gov/pesticides/op/ddvp.htm

U.S. EPA/ORD (U.S. Environmental Protection Agency/Office of Research and Development). 1992. Dermal Exposure Assessment: Principles and Applications. EPA/600/8-91/011B. Interim Report. Exposure Assessment Group, Office of Health and Environmental Assessment, Washington, DC. Available at: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=12188

U.S. EPA (U.S. Environmental Protection Agency, Office of Research and Development). 1993. Wildlife Exposure Factors Handbook. Volumes 1 and 2. EPA/600/R-93/187a,b. Pagination not continuous. Available NTIS: PB94-174778 and PB94-174779.

U.S. EPA (U.S. Environmental Protection Agency/Office of Research and Development). 1996. Exposure Factors Handbook. National Center for Environmental Assessment, U.S. EPA, Washington, DC. EPA/600/P-95/002Ba-c. Avail. NTIS: PB97-117683, 97-117691, PB97-117709.

USDA (U.S. Department of Agriculture). 1995a. Gypsy Moth Management in the United States: A Cooperative Approach. Final Environmental Impact Statement. Appendix F (Human Health Risk Assessment).

USDA (U.S. Department of Agriculture). 1995b. Gypsy Moth Management in the United States: A Cooperative Approach. Final Environmental Impact Statement. Appendix G (Risk Assessment).

USDA (U.S. Department of Agriculture). 2001. Gypsy Moth Program Manual. Prepared by Plant Protection and Quarantine, Animal and Plant Health Protection Service. Draft dated May, 2001. Available at: http://www.aphis.usda.gov/ppq/manuals/online_manuals.html.

Vadhva P; Hasan M. 1986. Organophosphate dichlorvos induced dose-related differential alterations in lipid levels and lipid peroxidation in various regions of the fish brain and spinal cord. J Environ Sci Health. B21(5): 413-424. (As cited by WHO 1989).

Venkat JA; Shami S; Davis K; Nayak M; Plimmer JR; Pfeil R; Nair PP. 1995. Relative Genotoxic activities of pesticides evaluated by a modified SOS microplate assay. Environ Mol Mut. 25 (1):67-76.

Vent-Axia Inc. 2004. Ventilation Requirements. http://www.vent-axia.com/sharing/requirements.asp

Verma SR; Tonk IP. 1984. Biomonitoring of the contamination of water by a sublethal concentration of pesticides. A system analysis approach. ACTA Hydrochim Hydrobiol. 12(4): 399-499. (As cited by WHO 1989).

Verma SR; Rani S; Bansal SK; Dalela RC. 1980. Effects of the pesticides thiotox, dichlorvos, and carbofuran on the test fish *Mystus vittatus*. Water Air Soil Pollut. 13(2): 229-234. (As cited by WHO 1989).

Verma SR; Rani S; Bansal SK; Dalela RC. 1981a. Evaluation of the comparative toxicity of thiotox, dichlorvos, and carbofuran to two fresh water teleosts, *Ophiocephalus punctatus* and *Mystus vittatus*. ACTA Hydrochem Hydrobiol. 9(2): 119-129. (As cited by WHO 1989).

Verma SR; Rani S; Dalela RC. 1981b. Isolated and combined effects of pesticides on serum transaminases in *Mystus vittatus* (African catfish). Toxicol Lett. 8: 67-71. (As cited by WHO 1989).

Verma SR; Rani S; Dalela RC. 1981c. Pesticide-induced physiological alterations in certain tissues of a fish, *Mystus vittatus*. Toxicol Lett. 9: 327-332. (As cited by WHO 1989).

Verma SR; Rani S; Dalela RC. 1981d. Determination of the maximum acceptable toxicant concentration (MATC) and the safe concentration for certain aquatic pollutants. ACTA Hydrochim Hydrobiol. 9(3): 247-254. (As cited by WHO 1989).

Verma S; Bansal S; Gupta A; Pal N; Tyagi A; Bhatnagar M; Kumar V; Dalela R. 1982a. Bioassay trials with twenty-three pesticides to a fresh water teleost, *Saccobranchus fossilis*. Water Res. 16: 525-529. (As cited by WHO 1989).

Verma SR; Rani S; Dalela RC. 1982b. Indicators of stress induced by pesticides in *Mystus vittatus*: haematological parameters. Indian J Environ Health. 24(1): 58-64. (As cited by WHO 1989).

Verma SR; Rani S; Tonk IP; Dalela RC. 1983. Pesticide-induced dysfunction in carbohydrate metabolism in three fresh water fishes. Environ Res. 32: 127-133. (As cited by WHO 1989).

Verma SR; Rani S; Dalela RC. 1984. Effects of pesticides and their combinations on three serum phosphatases of *Mystus vittatus*. Water Air Soil Pollut. 21: 9-14. (As cited by WHO 1989).

Vial T; Nicolas B; Descotes J. 1996. Clinical Immunotoxicity of Pesticides. J Toxicol Environ Health. 48 (3):215-229.

Vigliani EC. 1971. Exposure of newborn babies to VAPONA insecticide. Toxicol Appl Pharm. 19:379-380.

Voccia I; Blakley B; Brousseau P; Fournier M. 1999. Immunotoxicity of pesticides: A review. Toxicol Indus Health. 15(1-2):119-132.

Vogin EE; Carson S; Slomka MB. 1971. Teratology studies with dichlorvos in rabbits (Abstract No. 42). Toxicol Appl Pharmacol. 19:377-378.

Wagner JE; Johnson DR. 1970. Toxicity of dichlorvos for laboratory mice — LD_{50} and effect on serum cholinesterase. Lab Anim Care. 20:45.

Wallach JD; Frueh R. 1968. Pilot study of an organophosphate anthelmintic in camels and primates. J Am Vet Med Assoc. 153(7):798-799.

Weis N; Stolz P; Krooss J; Meierhenrich U. 1998. Dichlorvos insect strips indoors: Pollution and risk assessment. Gesundheitswesen. 60(7):445-9. (Publication is in German with an English abstract.)

WHO (World Health Organization). 1988. Dichlorvos Health and Safety Guide. Geneva, Switzerland: World Health Organization. Health and Safety Guide. 18:1-157.

WHO (World Health Organization). 1989. Environmental health criteria for dichlorvos. Geneva, Switzerland: World Health Organization. Environmental Health Criteria 79:1-157.

Wills JH. 1972. The measurement and significance of changes in cholinesterase activities of erythrocytes and plasma in man and animals. CRC Critical Reviews in Toxicology. March, pp. 153-202.

Wyrobek AJ, Bruce WR. 1975. Chemical induction of sperm abnormalities in mice. Proc Natl Acad Sci USA. 72(11):4425-4429.

Yamanoi F. 1980. [Effect of insecticides on the progeny in the silkworm *Bombyx mori*: I. Effect of organophosphorus insecticides on egg laying and their hatching.] Nippon Sanshigaku Zasshi. 49(5): 434-439 (in Japanese). (As cited by WHO 1989).

Yamashita M; Yamashita M; Tanaka J; Ando Y. 1997. Human mortality in organophosphate poisoning. Vet Human Toxicol. 39(2):84-85.

Zavon MR; Kindel EA. 1966. Potential hazard in using dichlorvos resin insecticide. Advances in Chemistry Series. 60:177-186.

Table 2-1: Selected physical and chemical properties of DDVP

Synonyms and trade names	SD 1750; Astrobot; Atgard; Canogard; Dedevap; Dichlorman; Dichlorophos; Dichlorvos; Divipan; Equigard; Equigel; Estrosol; Herkol; Nogos; Nuvan: Task; Vapona; Verdisol (Budavari 1989)
U.S. EPA Reg. No.	8730-50 (Hercon 2004)
CAS number	62-73-7 (ARS/PPD 1995; Meylan and Howard 2000)
Molecular weight	220.98 (Budavari 1989)
Molecular formula	$C_4H_7Cl_2O_4P$ (ARS/PPD 1995; Budavari 1989; Meylan and Howard 2000)
SMILES Notation	O=P(OC)(OC)OC=C(CL)CL (Meylan and Howard 2000)
Appearance/state, ambient	Liquid (ARS/PPD 1995; Budavari 1989)
mg/L to ppm conversion for air concentrations	1 ppm = 9.04 mg/m^3 (NOISH 2002) 1 mg/m^3 = 0.11 ppm
Boiling point	120°C at 14 mm Hg (ARS/PPD 1995) 251.76 °C (Meylan and Howard 2000)
Vapor pressure	1.2×10^{-2} mm Hg (Budavari 1989) 1,600 mPa (ARS/PPD 1995)
Water solubility (mg/L)	10,000 (Budavari 1989) 8,000 (ARS/PPD 1995)
Specific gravity	1.44 (Shell Chemical Company 1972)
log K_{ow}	1.40-2.29 (ARS/PPD 1995) [i.e., $K_{ow} = 10^{1.4} = 25.1$] 0.60 (estimated) (Meylan and Howard 2000) 1.47 (experimental) (Meylan and Howard 2000; U.S. EPA 1992)
Henry's law constant	0.044 Pa m^3/mole at 20 °C (ARS/PPD 1995) 8.58E-007 atm-m^3/mole (Meylan and Howard 2000)
Koc	40.2 (Meylan and Howard 2000)
BCF	0.4486 (Meylan and Howard 2000)
Hydrolysis half-time (days)	0.022 to 0.347 (ARS/PPD 1995)
Aqueous photolysis halftime (days)	2.295 (ARS/PPD 1995)

Table 3-1. Common effects of acetylcholinesterase inhibition [a]

System	Receptor Type	Organ	Action	Manifestation
Parasympathetic	Muscarinic	**Eye**		
		Iris muscle	Contraction	Miosis
		Ciliary muscle		Blurred vision
		Glands		
		Lacrimal	Secretion	Tearing
		Salivary		Salivation
		Respiratory		Bronchorrhea; rhinitis; pulmonary edema
		Gastrointestinal		Nausea; vomiting; diarrhea
		Sweat		Perspiration
Sympathetic (sympatholytic)		**Heart**		
		Sinus node	Slowing	Bradycardia
		Atrioventricular (AV) node	Increased refractory period	Dysrhythmia; heart block
		Smooth Muscle		
		Bronchial	Contraction	Broncho-constriction
		Gastrointestinal		Vomiting; cramps; diarrhea
				Fecal incontinence
		Sphincter	Relaxation	
		Bladder		
		Fundus	Contraction	Urination
		Sphincter	Relaxation	Urinary incontinence
Neuromuscular	nicotinic	**Skeletal**	Excitation	Fasciculations; cramps followed by weakness; pupillary dilation; loss of reflexes; paralysis
		Heart	Excitation	Tachycardia
Central nervous		**Brain/Brainstem**	Excitation (early)	Headache; malaise; dizziness; confusion; manic or bizarre behavior
			Depression (late)	Depression, then loss of consciousness; respiratory depression; respiratory (diaphragm) paralysis

[a] Modified from ATSDR 1993

Table 3-2: Parameters used in DDVP air model

Parameter	Value	Units	Description/Comment/Reference
$\tilde{a}$	37.5	Unitless	Apparent adsorption coefficient based on optimization using relative errors. See Worksheet A02b and Section 3.2.2.2 for discussion.
$\ddot{e}$	0.023	day^{-1}	First-order release rate from Shell No-Pest Strips from Gillett et al. (1972a). Used to estimate $\tilde{a}$ from the data reported by Slomka (1970).
	0.04	day^{-1}	First-order release rate from VaporTape II strips based on data from Hercon (1994). See Worksheet A01.
RH	0.4	Unitless	Relative humidity used by Gillett et al. (1972a) and used for model application in Worksheets A02a, A02b, A03a, and A03b. This is a sensitive parameter. See text for discussion.
k	109.3	days^{-1}	Hydrolysis rate constant from Gillett et al. (1972a)
At/*Va*	0, 60, and 625, and 6500	day^{-1}	Air turnover rate – i.e., the ratio of air flow to room volume. Values of 0 and 60 used by Gillett et al (1972a) for no ventilation and very poor ventilation, respectively. Values of 300 and 3000 are selected as adequate ventilation for a garage and vehicle, respectively – see Section 4.4 for discussion.

Table 3-3: Summary of Risk Characterization for Human Health Risk Assessment [1]

Group Scenario	Hazard Quotients					
	Central	Lower	Upper	Toxicity Value	Units	Section
Workers						
Inhalation During Assembly	**3**	0.9	**5**	0.1	mg/m^3	3.3.2.3
Inhalation During Transport	**15**	1.0	**18**	0.1	mg/m^3	3.3.2.3
Dermal During Assembly	0.5	0.2	**3**	0.0017	mg/kg	3.3.2.2
Child						
Incidental Dermal Contact	**10**	**1.8**	**60**	0.0017	mg/kg	3.3.2.2
Oral Exposure from Strip	**97**	**24**	**380**	0.0017	mg/kg	3.3.2.1
Oral Exposure from Water	0.008	0.002	0.04	0.0017	mg/kg	3.3.2.1

[1] All of the exposure assessments on which these hazard quotients are based should be regarded as atypical and most are extreme. As noted in Section 3.2, typical exposures for workers and members of the general public will typically be negligible.

Table 4-1: Summary of Exposure Assessments and Risk Characterization for Non-target Species

Exposure Assessments

			Estimated Exposures			
Species	**Scenario**	**Central**	**Lower**	**Upper**	**Units**	**Worksheet**
Racoon	Consumption	3.16E+01	1.05E+01	1.05E+02	mg/kg	D01 as DDVP-PVC
Small mammal	Contaminated Water	2.59E-05	8.64E-06	8.64E-05	mg/kg	D02 as free DDVP
Aquatic Species	Contaminated Water	0.000177	0.000059	0.00059	mg/L	D02

Risk Characterization

		Risk Quotients[1]			Toxicity Value	
Species	**Scenario**	**Central**	**Lower**	**Upper**	**Value**	**Units**
Racoon	Consumption	0.1	0.04	0.4	240	mg/kg as DDVP-PVC
Small mammal	Contaminated Water	0.0001	0.00002	0.0002	0.5	mg/kg as free DDVP
Aquatic Species						
	Fish	0.006	0.002	0.02	0.03	mg/L NOEC as free DDVP
	Sensitive Invertebrates	**3**	0.8	**8**	0.00007	mg/L LC_{50} as free DDVP
	Tolerant Invertebrates	0.00001	0.000003	0.00003	21	mg/L LC_{50} as free DDVP

[1] Risk quotients are calculated as the exposure value, given in the upper section of the table divided by the toxicity value specified for the non-target species. This ratio is rounded to one significant digit.

Figure 3-1: Concentration of DDVP in Air After the Placement of One Shell No-Pest Strip in an Unventilated Room (At/Va=0) and a Poorly Ventilated Room (At/Va=60)(data from Slomka 1970). See text for discussion and Worksheet A02b for details.

Appendix 1: Application and Optimization of DDVP Inhalation Exposure Model

Gillett et al. (1972a) proposed the following model for estimating concentrations of DDVP in air from the release of DDVP from PVC pest strips:

$$C_t = \frac{8}{\pi^2} \frac{M_0}{Va(1 + \gamma)} \frac{\exp(-\lambda t) - \exp(\frac{-(kRH + \frac{At}{Va})}{1 + \gamma} t)}{\frac{(kRH + \frac{At}{Va})}{\lambda (1+\gamma)} - 1}$$ (Eq. A-1)

The terms in the above equation are defined as follows:

t	time after start of release
C_t	concentration of DDVP in air at time, *t* (days)
M_0	mass of DDVP in strip or strips at time zero (mg)
Va	volume of room or other space (m^3)
ã	apparent adsorption coefficient of DDVP on to surfaces
exp(*x*)	the exponential function, e^x, where is the constant 2.718 and *x* is any numeric expression
ë	first-order release rate constant (days^{-1})
RH	relative humidity (proportion)
At	air flow rate (m^3/day)
k	first-order hydrolysis rate (days^{-1})

and the parameters used in the model are summarized in Table 3-2.

The above equation is modified from Equation 3 in Gillett et al. (1972, p. 126). For simplicity, the term *RH* is used above rather than the term p/p$_0$ used by Gillett – i.e., the ratio of the ambient to the saturated vapor concentration of water. More significantly, the equation given in the Gillett publication – i.e., Equation 3, p. 126 – contains two typographical errors. Both errors are in the numerator to the second exponential function. The Gillett publication fails to note that the negative of the sum, *k RH + At/Va*, must be used. These are essentially two first order processes – i.e., hydrolysis and dilution. If the negative of these values is not used, the equation models first-order growth rather than dissipation. Dissipation is clearly the intent of this term in the equation. The second more trivial error is that the *k RH + At/Va* term must be multiplied by *t* within the second exponential term. Otherwise, the units of the equation do not reduce to a concentration in air. This is analogous to the general equation for first-order absorption and first-

order elimination (e.g., Goldstein et al. 1974, p. 333). The discussion of the validation of this equation by Gillett et al. (1972a) and the implementation of this equation in the Worksheets uses the corrected form of the equation given above. Using the equation given by Gillett et al. (1972a) does not reproduce the results illustrated in Figure 4 of Gillett et al. (1972, p. 128) or in Worksheets A02a and A02b.]

Gillett et al. (1972a) applied this model to the data from Slomka (1970) in which a single Shell No-Pest Strip containing 20,000 mg of DDVP was placed rooms with a volume of 28.3 m^3 at 25°C and a relative humidity of 40%. Two different ventilation conditions were used, no ventilation and poor ventilation. No ventilation is characterized simply as a room with no air turnover – i.e., At/Va = 0. Poor ventilation is characterized as a room in which 20 air exchanges occurred per day – i.e., At/Va = 20. The apparent adsorption coefficient (ã) was treated as an empirical parameter and optimized to the data from Slomka (1970). All other model parameters were taken from the literature as specified in Table 3-2.

Gillett et al. (1972a) report an optimized value of 44.76 for the apparent adsorption coefficient (ã) but do not specify how this parameter was optimized. For the current risk assessment, the model given above was implemented in EXCEL and the data from Slomka (1970) was taken from Figure 4 in the publication of Gillett et al. (1972a). The apparent adsorption coefficient was then optimized using the EXCEL Solver function with the quasi-Newton method (with the tangent estimate and forward derivative options). Two sets of optimizations were conducted. The first was based on minimizing the standard square of error (Worksheet A02a) and the second was based on square of the relative error (Worksheet A02b). These optimizations yielded estimates of the apparent adsorption coefficient (ã) of 54.5 and 37.5, respectively, which bracket the estimate of 44.76 reported by Gillett et al. (1972a). As illustrated in Worksheets A02a and A02b, both of the optimized values fit the data from Slomka (1970) reasonably well. For the current risk assessment, the worker exposure estimates are based on the apparent adsorption coefficient (ã) 37.5, which leads to modestly higher estimates of exposure than do the higher estimates of the apparent adsorption coefficient. The fit of the Gillett et al. (1972a) model to the data from Slomka (1970) using the apparent adsorption coefficient (ã) of 37.5 is illustrated in Figure 3-1 (which is in turn taken from Worksheet A02b).

Appendix 2: Estimates of dermal absorption rates for DDVP

Table A2-1: Method for estimating the dermal permeability (K_p in cm/hr) and 95% confidence intervals.

Model parameters	ID	Value	
Coefficient for $k_{o/w}$	C_KOW	0.706648	
Coefficient for MW	C_MW	0.006151	
Model Constant	C	2.72576	
Number of data points	DP	90	
Degrees of Freedom (d.f.)	DF	87	
Critical value of $t_{0.025}$ with 87 d.f.[a]	CRIT	1.96	
Standard error of the estimate	SEE	45.9983	
Mean square error or model variance	MDLV	0.528716	
Standard deviation of model (s)	MSD	0.727129	$MDLV^{0.5}$
X′X, cross products matrix	0.0550931	-0.0000941546	-0.0103443
	-0.0000941546	0.0000005978	-0.0000222508
	-0.0103443	-0.0000222508	0.00740677

[a]Mendenhall and Scheaffer, 1973, Appendix 3, Table 4, p. A31.

NOTE: The data for this analysis are taken from U.S. EPA (1992), Dermal Exposure Assessment: Principles and Applications, EPA/600/8-91/011B, Table 5-4, pp. 5-15 through 5-19. The U.S. EPA report does not provide sufficient information for the calculation of confidence intervals. The synopsis of the above analysis was conducted in STATGRAPHICS Plus for Windows, Version 3.1 (Manugistics, 1995) as well as Mathematica, Version 3.0.1.1 (Wolfram Research, 1997). Although not explicitly stated in the U.S. EPA report, 3 of the 93 data points are censored from the analysis because they are statistical outliers: [Hydrocortisone-21-yl]-hemipimelate, n-nonanol, and n-propanol. The model parameters reported above are consistent with those reported by U.S. EPA but are carried out to a greater number of decimal places to reduce rounding errors when calculating the confidence intervals. See notes to Worksheet A07a for details of calculating maximum likelihood estimates and confidence intervals.

Table A2-2: Calculation of dermal permeability rate (K_p) in cm/hour for DDVP.			
Parameters	Value	Units	Reference
Molecular weight	220.98	g/mole	
$K_{o/w}$ at pH 7	29.51	unitless	
$\log_{10} K_{o/w}$	1.47		
Column vector a for calculating confidence intervals (see Worksheet A07a for definitions.)			
a_1	1		
a_2	220.98		
a_3	1.47		
Calculation of $a' \cdot (X'X)^{-1} \cdot a$ - see Worksheet A07b for details of calculation.			
Term 1	0.0190806955		
Term 2	0.001157619		
Term 3	-0.006428795		
$a' \cdot (X'X)^{-1} \cdot a$	0.0138	calculation verified in Mathematica 3.0.1.1	

$\log_{10} k_p = 0.706648 \log_{10}(k_{o/w}) - 0.006151\, MW - 2.72576$							Worksheet A07b
$\log_{10}$ of dermal permeability							
Central estimate	-3.04623542	±	$t_{0.025}$	×	s	×	$a'\cdot(X'X)^{-1}\cdot a^{0.5}$
Lower limit	-3.21365532088	-	1.9600	×	0.727129	×	0.1174734012
Upper limit	-2.87881551912	+	1.9600	×	0.727129	×	0.1174734012
Dermal permeability							
Central estimate	0.00090	cm/hour					
Lower limit	0.00061	cm/hour					
Upper limit	0.0013	cm/hour					

Details of calculating $a'X'X\,a$

The term $a'\cdot(X'X)^{-1}\cdot a$ requires matrix multiplication. While this is most easily accomplished using a program that does matrix arithmetic, the calculation can be done with a standard calculator. See details on following page.

Appendix 2-2

Letting

$$\mathbf{a} = \{a\_1, a\_2, a\_3\}$$

and

$$(\mathbf{X'X})^{-1} = \quad \{$$

$$\{b\_1, b\_2, b\_3\},$$
$$\{c\_1, c\_2, c\_3\},$$
$$\{d\_1, d\_2, d\_3\}$$
$$\},$$

$\mathbf{a'}\cdot(\mathbf{X'X})^{-1}\cdot\mathbf{a}$ is equal to

Term 1:	$\{a\_1 \times([a\_1 \times b\_1] + [a\_2 \times c\_1] + [a\_3 \times d\_1])\} +$
Term 2:	$\{a\_2 \times([a\_1 \times b\_2] + [a\_2 \times c\_2] + [a\_3 \times d\_2])\} +$
Term 3:	$\{a\_3 \times([a\_1 \times b\_3] + [a\_2 \times c\_3] + [a\_3 \times d\_3])\}.$

Appendix 2-3

Appendix L
Gypsy Moth
Risk Assessment

Figure L-1. Gypsy moth caterpillars cluster at the base of a banded tree (Arlington, Virginia, 1905).

Control/Eradication Agents for the Gypsy Moth -
Human Health and Ecological Risk Assessment for the Gypsy Moth
FINAL REPORT

Prepared for:

USDA, Forest Service
Forest Health Protection

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Submitted to:
Dave Thomas, COTR
Forest Health Protection Staff
USDA Forest Service
Rosslyn Plaza Building C, Room 7129C
1601 North Kent Street
Arlington, VA 22209

Submitted by:
Patrick R. Durkin
Syracuse Environmental Research Associates, Inc.
5100 Highbridge St., 42C
Fayetteville, New York 13066-0950
Telephone: (315) 637-9560
Fax: (315) 637-0445
E-Mail: SERA_INC@msn.com
Home Page: www.sera-inc.com

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AEL	adverse-effect level
bw	body weight
B.t.k.	*Bacillus thuringiensis* var. *kurstaki*
CI	confidence interval
cm	centimeter
d.f.	degrees of freedom
EC_x	concentration causing X% inhibition of a process
EC_{25}	concentration causing 25% inhibition of a process
EC_{50}	concentration causing 50% inhibition of a process
F	female
FH	Forest Health
g	gram
ha	hectare
HQ	hazard quotient
kg	kilogram
L	liter
lb	pound
LdNPV	*Lymantria dispar* (gypsy moth) nuclear polyhedrosis virus
LOAEL	lowest-observed-adverse-effect level
LOC	level of concern
m	meter
M	male
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOS	not otherwise specified
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
RfD	reference dose
SERA	Syracuse Environmental Research Associates
UF	uncertainty factor
U.S.	United States
USDA	U.S. Department of Agriculture
U.S. EPA	U.S. Environmental Protection Agency
μ	micron or micro-

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

To convert ...	Into ...	Multiply by ...
acres	hectares (ha)	0.4047
acres	square meters (m²)	4,047
atmospheres	millimeters of mercury	760
centigrade	Fahrenheit	1.8 °C+32
centimeters	inches	0.3937
cubic meters (m³)	liters (L)	1,000
Fahrenheit	centigrade	0.556 °F-17.8
feet per second (ft/sec)	miles/hour (mi/hr)	0.6818
gallons (gal)	liters (L)	3.785
gallons per acre (gal/acre)	liters per hectare (L/ha)	9.34
grams (g)	ounces, (oz)	0.03527
grams (g)	pounds, (oz)	0.002205
hectares (ha)	acres	2.471
inches (in)	centimeters (cm)	2.540
kilograms (kg)	ounces, (oz)	35.274
kilograms (kg)	pounds, (lb)	2.2046
kilograms per hectare (hg/ha)	pounds per acre (lb/acre)	0.892
kilometers (km)	miles (mi)	0.6214
liters (L)	cubic centimeters (cm³)	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 (cm³)	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/m²)	112.1
pounds per acre (lb/acre)	µg/square centimeter (µg/cm²)	11.21
pounds per gallon (lb/gal)	grams per liter (g/L)	119.8
square centimeters (cm²)	square inches (in²)	0.155
square centimeters (cm²)	square meters (m²)	0.0001
square meters (m²)	square centimeters (cm²)	10,000
yards	meters	0.9144

Note: All references to pounds and ounces refer to avoirdupois weights unless otherwise specified.

CONVERSION OF SCIENTIFIC NOTATION

Scientific Notation	Decimal Equivalent	Verbal Expression
$1 \cdot 10^{-10}$	0.0000000001	One in ten billion
$1 \cdot 10^{-9}$	0.000000001	One in one billion
$1 \cdot 10^{-8}$	0.00000001	One in one hundred million
$1 \cdot 10^{-7}$	0.0000001	One in ten million
$1 \cdot 10^{-6}$	0.000001	One in one million
$1 \cdot 10^{-5}$	0.00001	One in one hundred thousand
$1 \cdot 10^{-4}$	0.0001	One in ten thousand
$1 \cdot 10^{-3}$	0.001	One in one thousand
$1 \cdot 10^{-2}$	0.01	One in one hundred
$1 \cdot 10^{-1}$	0.1	One in ten
$1 \cdot 10^{0}$	1	One
$1 \cdot 10^{1}$	10	Ten
$1 \cdot 10^{2}$	100	One hundred
$1 \cdot 10^{3}$	1,000	One thousand
$1 \cdot 10^{4}$	10,000	Ten thousand
$1 \cdot 10^{5}$	100,000	One hundred thousand
$1 \cdot 10^{6}$	1,000,000	One million
$1 \cdot 10^{7}$	10,000,000	Ten million
$1 \cdot 10^{8}$	100,000,000	One hundred million
$1 \cdot 10^{9}$	1,000,000,000	One billion
$1 \cdot 10^{10}$	10,000,000,000	Ten billion

EXECUTIVE SUMMARY

OVERVIEW

The best documented and most obvious effect of the gypsy moth will be on terrestrial vegetation, particularly forest stands in which sensitive species of trees predominate. In sensitive forest stands, gypsy moth larvae can cause substantial defoliation. In forest stands in which tree species that are not favored by gypsy moth larvae predominate, even relatively high exposures may not result in substantial defoliation.

The gypsy moth may also have a direct impact on human health and the most likely effects will involve skin irritation. In heavy gypsy moth infestations, adverse skin reactions would be expected in substantial numbers and the effects would likely be sufficiently severe to cause some individuals to seek medical attention. In extreme outbreaks, the effects will be qualitatively similar to those of severe infestations but could affect up to about one-third of the human population.

Because the gypsy moth may substantially damage some forests in severe infestations or outbreaks, secondary effects in some species of wildlife are plausible and include reductions in populations of squirrels, mice, and other mammals which may be sensitive to changes in the availability of acorns are likely. Substantial adverse effects on other groups of animals – i.e., birds, reptiles, and aquatic species – cannot be ruled out but have not been convincingly demonstrated.

GYPSY MOTH AS A PEST SPECIES

The gypsy moth is a pest species that can cause substantial damage to some forests. In the eastern United States, most hardwood forests are classified as susceptible to gypsy moth infestation and as many as 12.5 million acres have been defoliated in a single season. The gypsy moth is found throughout much of New England and south to Virginia and west to portions of Wisconsin. The potential for substantial outbreaks is often assessed based on counts of overwintering egg masses, which are relatively easy to measure and can be made in time to plan for and take preventative measures against the outbreak.

The life cycle of the gypsy moth consists of the egg, larval, pupal, and adult stages with one generation produced each year. The larvae or caterpillars go through various sub-stages, referred to as instars. First stage larvae (first instars) hatch in early to late May and go through additional larval stages between May and late June. First instars spin fine silk threads near the tops of trees from which they suspend themselves; in the event of sufficient wind, these threads break allowing the caterpillars to be transported by the wind. The distances involved in wind dispersion may cover several miles. Between late June and mid-July, the caterpillars spin sparse silken cradles and form pupae. After 7–14 days of pupation, the adult moths emerge and mate, and the life cycle is repeated.

The gypsy moth is susceptible to diseases caused by gypsy moth pathogens like *B.t.k.*, the gypsy moth nuclear polyhedrosis virus (LdNPV), and *Entomophaga maimaiga* fungi. *B.t.k.* and LdNPV are also used as control agents for the gypsy moth and these agents are addressed individually in separate risk assessments. The gypsy moth is a prey species for some mammals, birds, and other insects. In general, invertebrates are the major predators of gypsy moth larvae, while small mammals are the major predators of pupae. Numerous insects, including the larvae of various flies and wasps, act as parasites or predators to the gypsy moth.

HUMAN HEALTH RISK ASSESSMENT

Hazard Identification – Skin irritation after contact with larvae of many species of lepidoptera is common and this effect is the most common and best documented response to contact with gypsy moth larvae. The skin reactions seem to be associated with contact with small fine hairs that stick out from the body of the larva. The precise mechanism or mechanisms of action for these irritant effects is unclear but may involve three general responses: mechanical irritation, toxic reaction to a compound such as histamine, and an immediate or delayed allergic reaction. Raised and reddened areas of skin, known as wheals, are the most characteristic skin lesions. These lesions, resembling the raised patches of skin often associated with mosquito bites, may be approximately 0.25–0.5 inches in diameter. Contact with larvae may also cause rashes rather than wheals. Both wheals and rashes may cause severe itching that can persist for several days to 2 weeks and may be sufficiently severe to cause the affected individual to seek medical treatment. Other effects that may be associated with exposure to gypsy moth larvae include eye and respiratory irritation but these effects are less well documented, compared with dermal effects.

In very severe infestations, the large numbers of larvae in an area may cause stress or anxiety in some individuals. Also during heavy infestations, water quality may be affected by increased runoff and by direct contamination with frass. Nonetheless, there are no documented cases of changes in water quality being associated with adverse effects in humans.

Exposure Assessment – The number of larvae per unit area or tree might be considered the most direct and relevant measure of human exposure because it is contact with the larvae that causes skin irritation, the adverse effect typically associated with the gypsy moth. The available dose response data, however, are based on studies in which exposure is quantified as the number of eggs masses per acre and thus this is the exposure measure that is used in this risk assessment. As long as gypsy moth populations remain sparse, the larvae usually eat only a small proportion of the foliage of even their most favored host species, and contacts with people are rare. In such cases, egg masses generally do not exceed 50 egg masses/acre. During full-scale outbreaks, densities of about 5000 egg masses/acre are common and densities greater than 20,000 egg masses/acre are occasionally recorded.

Dose-Response Assessment – The dose-response assessment for human health effects is based on reports of skin irritation in two populations: one with low exposure (an average of 32 egg masses/acre) and the other with high exposure (an average of 3809 egg masses/acre). The low-

exposure group exhibited no increase in skin irritation and 32 egg masses/acre is taken as a NOAEL (no adverse effect level) for humans and is used as a surrogate RfD (reference dose) for exposure to the gypsy moth in a manner analogous to the use of RfD values for control agents. The high exposure group did evidence a significant increase in skin irritation and, based on a dose-response model developed by U.S. EPA, egg mass densities up to 128 egg masses/acre are not likely to cause a detectable increase in skin irritation or rashes. In addition to these quantitative estimates, the severity of the response is important, particularly in a comparison of effects caused by exposure to the gypsy moth and effects caused by exposure to the agents used to control the gypsy moth. Dermal responses to the gypsy moth are sufficiently severe to have generated numerous case reports. While precise statistics are not available, it does appear that the severity of the skin irritation is sufficient to cause appreciable numbers of affected individuals to seek medical care. While exposure to the gypsy moth is associated with irritation to the eyes and respiratory tract, quantitative dose-response relationships for these endpoints cannot be developed.

Risk Characterization – In sparse to moderate infestations—i.e., egg mass densities of <500 egg masses/acre—adverse effects involving skin irritation are not likely to be detectable in populations of exposed humans. Nonetheless, some individuals who come into contact with gypsy moth larvae could develop skin irritation. In heavy gypsy moth infestations—i.e., >500 to 5000 egg masses/acre—adverse skin reactions would be expected in substantial numbers and the effects would likely be sufficiently severe to cause some individuals to seek medical attention. In extreme outbreaks—i.e., >5,000 to 20,000 egg masses/acre— the effects will be qualitatively similar to those of severe infestations but could affect up to about one-third of the population. Heavy infestations or extreme outbreaks could cause ocular and respiratory effects in some people but the likelihood of observing these effects cannot be quantified. Similarly, severe infestations are often considered to be a nuisance and cause aesthetic damage to the environment. Both of these factors can lead to stress in some individuals. Young children may be a group at special risk from effects of gypsy moth exposure but it is not clear whether children are more sensitive than adults to the effects of gypsy moth exposure or whether responses in children appear greater because children spend more time outdoors compared with adults.

ECOLOGICAL RISK ASSESSMENT
Hazard Identification – The clearest primary effect of gypsy moth infestations is on terrestrial plants, primarily trees. Various instars of the gypsy moth larvae will feed on host trees and can cause extensive defoliation which can kill some of the infested trees. On a larger scale, the extensive defoliation and/or death of trees may result in secondary changes to vegetation, which will, in turn, affect other forms of vegetation as well as various animal species (primarily related to changes in habitat). Gypsy moth larvae appear to have definite food preferences; oak, birch, poplar, and apple trees seem to be their favorite food sources. While both the European and Asian gypsy moth cause similar types of damage (i.e., defoliation), their feeding preferences are somewhat different with the Asian gypsy moth preferring a wider range of vegetation. Heavy defoliation is much more common among the oaks than among trees that are not particularly favored as food by the gypsy moth. For susceptible oaks, the effects of infestations on tree

mortality varies according to the initial condition of the stand and the number of infestations. Generally, gypsy moth infestations result in mortality of less than 15% of total basal area – i.e., mortality of trees involving 15% the total area of the tree trunks near the ground. When heavy defoliation is followed by massive overstory mortality, existing shrub and herb cover increase dramatically due to increases in available light, moisture, and nutrients. Extensive loss of the existing canopy will also favor the growth of tree species that are intolerant to shade and will shift the forest ecosystem towards earlier successional stages.

The only other groups of organisms that are likely to be directly affected by the gypsy moth are some and probably very few other lepidopteran species, including the northern tiger swallowtail butterfly. The mechanisms for direct adverse effects on other lepidopteran species may include bacterial contamination of the leaves by gypsy moth larvae and a decrease in the nutritional value of the leaves damaged by the gypsy moth. Most studies, however, do not indicate substantial direct effects on other insects, including lepidoptera. In some cases, increases may be seen in populations of insect predators of the gypsy moth.

There is no indication in the literature that the gypsy moth will cause direct adverse effects in most groups of animals. Indirect effects, associated with damage to vegetation, may be of substantial consequence to some species, including squirrels, mice, and other mammals that rely on acorns. Although some mammals consume insects, including the gypsy moth, there is no evidence that gypsy moth outbreaks have a substantial impact on insectivorous mammals. Similarly, there is little indication that birds or aquatic species will be adversely affected by the gypsy moth. In some species of birds, gypsy moth infestations and subsequent defoliation may be beneficial, especially for species of birds that favor dead wood as a habitat.

Exposure Assessment – As in the human health risk assessment, the exposure metameter is dictated by the data used to formulate the dose-response assessment. Also as in the human health risk assessment, egg mass density is the exposure metameter for terrestrial invertebrates and plants because it is the measure on which the dose-response assessment is based. Egg mass densities spanning a range from 5 egg masses/acre to 5,000 egg masses per acre are used to estimate responses in terrestrial plants and invertebrates.

Most wildlife species are not affected directly by exposure to the gypsy moth but are more likely to experience indirect effects like changes in habitat or other environmental conditions secondary to defoliation. Consequently, the exposure assessment for most wildlife species is almost identical to the dose-response assessment for terrestrial plants which is expressed as defoliation caused by gypsy moth larvae. For this exposure assessment, categories of defoliation are defined normal background defoliation (<30% defoliation), moderate defoliation (30-60% defoliation), and high or severe defoliation (>60% defoliation).

Dose-Response Assessment – As in the human health risk assessment, the dose metameter is egg masses/acre. Quantitative dose-response assessments can be made for both terrestrial plants and sensitive species of lepidoptera. The dose-response assessments for terrestrial plants are based

on a relatively simple quantitative model for the relationship of egg mass density to defoliation. Three broad categories (sensitive, intermediate, and tolerant) are used to characterize the susceptibility of forest stands to gypsy moth induced defoliation. Estimated LOAEL values based on 30% defoliation, which is considered the lower range of moderate defoliation, are approximately 125, 1000, and 7000 egg masses/acre for sensitive, intermediate, and tolerant forest stands, respectively. The corresponding NOAEL values, defined as 10% defoliation, are estimated as 12, 20, and 125 egg masses/acre for sensitive, intermediate, and tolerant forest stands.

The effects of gypsy moth exposure on sensitive terrestrial invertebrates, including some species of lepidoptera, are less well documented and less well characterized, compared with the effects on terrestrial plants. Nonetheless, available studies indicate that the NOAEL for adverse effects in certain other species of lepidoptera are lower than the NOAEL for sensitive forest stands—— i.e., about 6-72 egg masses/acre for some lepidoptera.

No quantitative dose-response assessment is presented for other groups of organisms—e.g., mammals, birds, and soil or aquatic organisms. The impact of gypsy moth exposure on these species is most likely to result in indirect effects secondary to defoliation.

Risk Characterization – The best documented and most obvious effect of the gypsy moth will be on terrestrial vegetation, particularly forest stands in which sensitive species of trees predominate. In some respects, the risk characterization for terrestrial vegetation is essentially a restatement of the hazard identification. In other words, the effects of gypsy moth larvae on forests is extremely well documented and relatively well understood. In sensitive forest stands—i.e., stands in which oak, birch, and other favored species predominate —gypsy moth larvae can cause substantial defoliation. In forest stands in which tree species that are not favored by gypsy moth larvae predominate —e.g., hemlock, various types of pine, black locust and white ash—even relatively high exposures, as measured by egg mass density, may not result in substantial defoliation. The risk assessment for direct effects on forests should be at least qualitatively influenced by the current range of the gypsy moth, which has not yet extended to some forests in the southeast that may be among the most sensitive to gypsy moth exposure. Thus, unless measures to contain the gypsy moth are successful, the southeastern oak forests may suffer serious damage in future infestations.

Some other lepidopteran species also may be directly affected by exposure to the gypsy moth. Most studies, however, suggest that substantial adverse effects in terrestrial insects are unlikely and effects in some insect species, including some other lepidoptera, may be beneficial.

Because the gypsy moth may substantially damage some forests in severe infestations or outbreaks, secondary effects in other species of wildlife are plausible. Reductions in populations of squirrels, mice, and other mammals which may be sensitive to changes in the availability of acorns are likely. Substantial adverse effects on other groups of animals – i.e., birds, reptiles, and aquatic species – cannot be ruled out but have not been convincingly demonstrated.

1. INTRODUCTION

This report addresses the potential human health effects and ecological effects of gypsy moth infestations and is part of the effort to update the 1995 Final Environmental Impact Statement (FEIS) for the Cooperative Gypsy Moth Management Program. The effort to update the FEIS involves the preparation of human health risk assessments (HHRAs) and ecological risk assessments (ERAs) for each of the agents used to control or eradicate gypsy moth infestations: *Bacillus thuringiensis kurstaki* (*B.t.k.*), Gypchek, diflubenzuron, tebufenozide, DDVP and disparlure. This risk assessment of the gypsy moth is intended to assist the USDA in assessing the consequences of "no action" alternatives in the FEIS. In addition, a separate document in this series will compare the effects gypsy moth infestations with the effects of the agents used to control the infestations.

This documents consists of an introduction, an overview of the gypsy moth as a pest species (Section 2), a risk assessment for human health effects (Section 3), and a risk assessment for ecological effects or effects on non-target wildlife species (Section 4). Each of the two risk assessment chapters has four major sections, including an identification of the hazards associated with the gypsy moth, an assessment of potential exposure to the gypsy moth, an assessment of the dose-response relationships, and a characterization of the risks associated with plausible levels of exposure. These are the basic steps recommended by the National Research Council of the National Academy of Sciences (NRC 1983) for conducting and organizing risk assessments.

The risk assessment on the gypsy moth is different from the risk assessments for chemical and biological agents used to control gypsy moth infestations, primarily because many standard physical and chemical properties used to characterize control agents and estimate certain exposure parameters are not at issue. Moreover, estimates of human and ecological exposure to all control agents—chemical and biological— are based on application rates, (i.e., known amounts of the agent applied under reasonably well defined conditions), which are not relevant to the gypsy moth. As discussed in subsequent sections of this document, estimates regarding gypsy moth exposure are extremely variable and difficult to define.

A tremendous body of information is available on the biology, physiology, and population dynamics of the gypsy moth and this information is presented in reviews, books, and monographs that are available in the open literature (e.g., Davidson et al. 1999, 2001; Gansner et al. 1993a; Gerardi and Grimm 1979; Herrick and Gansner 1988; Liebhold 1992; Nealis et al. 1999; Sharov et al. 1999, 2002; Wallner 1994, 1996; Williams et al. 2000). Additional information on the gypsy moth is available at a USDA Forest Service web site, http://na.fs.fed.us/wv/gmdigest/. The current risk assessment makes no attempt to summarize all of this information. Although some background information is presented (Section 2), the primary focus of this document is on the information that can be used directly to assess the human health effects (Section 3) and ecological effects (Section 4) of the gypsy moth in ways that correspond to and may be compared to the risk assessments of agents used to control or eradicate gypsy moth infestations.

This is a technical support document that addresses some specialized technical areas. Nevertheless, an effort was made to ensure that the document can be understood by individuals who do not have specialized training in the chemical and biological sciences. Certain technical concepts, methods, and terms common to most risk assessments are described in a separate document (SERA 2001). In addition, general glossaries of environmental terms are widely available and a custom glossary designed to be used in conjunction with USDA risk assessments is available at www.sera-inc.com. Some of the more complicated terms that are specific to the gypsy moth are defined in the text of this risk assessment.

2. GYPSY MOTH AS PEST SPECIES

2.1. OVERVIEW

The gypsy moth is a pest species that can cause substantial damage to some forests. In the eastern United States, most hardwood forests are classified as susceptible to gypsy moth infestation and as many as 12.5 million acres have been defoliated in a single season. The gypsy moth is found throughout much of New England and south to Virginia and west to portions of Wisconsin. The potential for substantial outbreaks is often assessed based on counts of overwintering egg masses, which are relatively easy to measure and can be made in time to plan for and take preventative measures against the outbreak.

The life cycle of the gypsy moth consists of the egg, larval, pupal, and adult stages with one generation produced each year. The larvae or caterpillars go through various sub-stages, referred to as instars. First stage larvae (first instars) hatch in early to late May and go through additional larval stages between May and late June. First instars spin fine silk threads near the tops of trees from which they suspend themselves; in the event of sufficient wind, these threads break allowing the caterpillars to be transported for long distances by the wind. Between late June and mid-July, the caterpillars spin sparse silken cradles and form pupae. After 7–14 days of pupation, the adult moths emerge and mate, and the life cycle is repeated.

The gypsy moth is susceptible to diseases caused by gypsy moth pathogens including *B.t.k.*, the gypsy moth nuclear polyhedrosis virus (LdNPV), and *Entomophaga maimaiga* fungi. *B.t.k.* and LdNPV are also used as control agents for the gypsy moth and these agents are addressed individually in separate risk assessments. The gypsy moth is a prey species for some mammals, birds, and other insects. In general, invertebrates are the major predators of gypsy moth larvae, while small mammals are the major predators of pupae. Numerous insects, including the larvae of various flies and wasps, act as parasites or predators to the gypsy moth.

2.2. INFESTATIONS

The current scientific name is for the gypsy moth is *Lymantria dispar*. In the older literature (e.g., Gerardi and Grimm 1979), the gypsy moth is referred to by its previous scientific name, *Porthetria dispar*. Over three quarters of the hardwood forests in the eastern United States are classified as susceptible to the gypsy moth (USDA/FS 1990). In addition, many forests in the south and central regions of the country, currently beyond the range of the gypsy moth, are likely to be very susceptible to damage by the gypsy moth (Liebhold and McManus 1999). In a major outbreak, the extent of damage can be substantial and as many as 12.5 million acres have been defoliated in a single season (Williams 1982). Damage to vegetation is caused by feeding larvae. During outbreaks, gypsy moth larval populations may range from about 10,000 to 250,000 larvae per hectare (Colbert et al. 1995; Christie et al. 1995).

The gypsy moth was brought into the United States intentionally in 1869 as part of an experiment by a naturalist, Leopald Trouvelet, to develop a hardy silk-producing insect. In the course of the experiments, conducted in Medford, Massachusetts, some gypsy moth eggs were lost and a

population of gypsy moths was established in the Medford area. The gypsy moth population grew to infest about a 400 square mile area around Medford by 1880, and the first major outbreak occurred in 1889 (Gerardi and Grimm 1979). The gypsy moth has spread throughout much of New England and south to Virginia and east to portions of Wisconsin. Current and plausible future infestations are discussed further in the exposure assessment for human health effects (Section 3.2) and ecological effects (Section 4.2). Figure 2-1 summarizes information regarding the frequency of gypsy moth defoliations over a period of 28 years—i.e., 1975 to 2002. In any given year, marked defoliations associated with gypsy moth infestations may be less ubiquitous and may be isolated in relatively small areas, which is due both to the control measures taken to limit gypsy moth populations as well as to the natural variability in gypsy moth populations.

The population pattern observed after the release of the first gypsy moths in North America—i.e., a period of low and inconsequential population growth followed by a major outbreak—is typical of gypsy moth population dynamics, which are described as bimodal (i.e., existing either at innocuous densities or in an outbreak or very rapid growth mode) (Campbell 1981).   Following an initial outbreak, populations generally decline and are usually maintained at low population densities that cause little damage. Subsequent outbreaks are usually less severe than the initial outbreak. As discussed further in Section 4.2, gypsy moth outbreaks are often associated with the presence of favored tree species (Baker and Cline 1936; Behre 1939; Behre and Reineke 1943). In general, gypsy moth outbreaks in North America dissipate in 1or 2 years. In rare cases, outbreaks can recur annually over periods of up to 20 years (Bess et al. 1947; Campbell 1973).

For at least half a century, the gypsy moth has persisted at generally innocuous densities in the predominantly oak forests of northeastern Connecticut and adjacent Massachusetts (Bess et al. 1947; Brown and Sheals 1944). During such intervals, gypsy moth larvae usually eat only a small proportion of the foliage of even their most favored host species. When defoliation is low, nearly all of it occurs on favored-food trees (Campbell and Sloan 1977b). Once a large-scale outbreak is underway, the gypsy moth will feed on a greater variety of vegetation and over 300 species of broadleaf and coniferous trees and shrubs may be damaged (Leonard 1981; Liebhold et al. 1994).

The potential for substantial outbreaks is often assessed based on counts of overwintering egg masses. Such counts are relatively easy to make and can be made in time to plan for and take preventative measures against a potential outbreak (Buss et al. 1999). Egg mass counts, however, are not absolute predictors of outbreak potential and egg masses per acre can be highly variable. In an infested area in Maryland, egg masses ranged from about 20/ha to 14,000/ha at 16 sites over a 4-year period (Davidson et al. 2001). In heavily infested areas, eggs masses per acre can range from about 5000 to 43,000 (Hajek 1997). The relationship of egg mass density to subsequent damage is complicated by the fact that the survival of egg masses is also highly variable, ranging from <1% to about 90% (Nealis et al. 1999). In areas with extremely cold winters, egg masses laid below the snow line tend to have higher survival rates than those laid above the snow line (Nealis et al. 1999; Smitley et al. 1998).   Bess (1961) reports that reduced

defoliation, which followed a winter of prolonged below zero temperatures, was due to 90% overwintering egg mortality.

The spread of an infestation may also be influenced by available vegetation. While some studies show that the quality of resources available to the female gypsy moth has only a minor effect on population dynamics (Erelli and Elkinton 2000), other sources indicate that the consumption of vegetation by larvae and subsequent larval growth may differ substantially according to vegetation type (Foss and Rieske 2003).

The spatial distribution of stand susceptibility is a key characteristic in the spread of outbreaks and subsequent defoliation (Liebhold and McManus 1991). Outbreaks are described as originating in small, discrete locations. These locations, referred to as foci, are usually characterized by stands growing on stressed sites like ridge tops, upper slopes, and deep sands, frequently subject to drought (Houston and Valentine 1977). These areas can support moderate to high populations of gypsy moth when the insect is undetectable in surrounding areas (Liebhold and McManus 1991). Protected resting locations that favor larval and pupal survival are known to support larger gypsy moth populations and lead to outbreaks (Bess et al. 1947; Campbell and Sloan 1977a; Houston 1975; Houston and Valentine 1977).

Other factors that may precipitate outbreaks include predator failure and specific climatic and meteorological conditions. Khanislamov and Girfanova (1964) demonstrate that weather variation may have more drastic effects on the natural enemies of gypsy moth than on the pest itself. Population collapse at the end of an outbreak appears to be the result of disease (Section 2.4), reduced fecundity, and starvation (Campbell 1981). Although dispersal of young larvae plays a role in gypsy moth outbreaks, it is thought to play a relatively minor role in outbreak initiation. Larval dispersion may be the major cause of gypsy moth distribution enlargement and range expansion at innocuous densities but it does not appear to cause outbreaks to spread (Campbell 1976). The rate at which infestations spread may vary substantially according to vegetation type and the methods used to control the spread. Reported rates of infestation range from 12 to 145 km/year (Sharov et al. 1999; Wallner 1996).

There are various models to predict the effects of gypsy moth infestations on a mixed hardwood forest (Colbert and Racin 1995; Colbert et al. 1995; Weseloh 1996a,b; Wilder et al. 1995; Williams et al. 1997). These models are discussed further in the dose-response assessment for terrestrial vegetation (Section 4.3.2).

2.3. LIFE-CYCLE
As with most insects, the life cycle of the gypsy moth consists of the egg, larval, pupal, and adult stages (Abrahamson and Klass 1982; Cram 1990; Gerardi and Grimm 1979). In the northeast, the adult female lays eggs in July or August. The larvae or caterpillars go through various stages, referred to as instars. First stage larvae (first instars) hatch in early to late May and go through additional larval stages (a total of five instars in males and six instars in females) between May and late June. This process occurs somewhat earlier in the southeast. The transition from each

stage to the next involves molting, during which time the caterpillar sheds its outer skin. It is during the larval stages that feeding occurs. First instars spin fine silk threads near the tops of trees from which they suspend themselves. After the thread breaks, the larvae can be transported over relatively long distances by the wind. The distances that larvae might be carried by wind is likely to be highly variable and has not been well or generally characterized. Gerardi and Grimm (1979, p. 63) note that larvae have been monitored at elevations of up to 2000 feet and have been found at distances of up to 35 miles from the closest known infestation. Between late June and mid-July, the caterpillars spin sparse silken cradles and form pupae. After 7–14 days of pupation, the adult moths emerge and mate, and the life cycle is repeated.

Newly hatched larvae often remain on the egg mass for several days before climbing toward foliage. First-instar gypsy moth larvae have two types of hairs or setae: long thin hairs that appear to assist the larvae in "soaring" or transport by wind and short hairs (sometimes referred to as "balloon hair") that contain chemicals like nicotine which may serve as a defense mechanism to discourage predators (Bardwell and Averill 1996; Deml and Dettner 1995; Smith 1985).

Moths begin to emerge about the middle of July, with males appearing several days earlier than females. In the south, moth emergence may occur as early as June. The European female cannot fly; she emits a pheromone (sex attractant) that volatilizes and is carried in the air. Male moths are attracted to the pheromone for distances up to 1 mile. After fertilizing and depositing eggs the adult moths do not eat and soon die (Johnson and Lyon 1988). Egg masses are deposited on tree trunks, rocks, and litter. Although the eggs overwinter, below normal temperatures can cause egg mortality (Bess 1961).

This risk assessment considers both the European and Asian gypsy moths, which are considered to be the same species (*Lymantria dispar*). Since the European gypsy moth was introduced in North America from closely related individuals, genetic studies indicate little variation within or between populations. The Asian gypsy moth, on the other hand, displays considerable variability within populations. The variability is expressed morphologically in the variety of larval color forms, behaviorally in the female flight capability, and physiologically in the capacity of larvae to colonize aggressively a broad spectrum of hosts (USDA/FS 1992).

While the female European gypsy moth is flightless, the Asian female is a strong flier capable of flights in excess of 18 miles (30 km) (USDA/FS 1992; Wallner 1996). Since the female Asian gypsy moth is able to lay eggs far from the pupal site following flight, this characteristic alone may make it necessary to modify the control methods of detection, delimitation, and control or eradication developed for the European gypsy moth (USDA/FS 1992). Asian gypsy moth larvae tend to feed more aggressively and on a broader variety of trees than their European counterpart (Wallner 1996).

2.4. DISEASE AND PREDATION

The primary focus of this risk assessment is effects of the gypsy moth on other species. Nonetheless, many organisms may adversely affect the gypsy moth, thereby reducing the risks posed by gypsy moth infestations. The gypsy moth is susceptible to diseases, including diseases caused by pathogens like *B.t.k.*, the gypsy moth nuclear polyhedrosis virus (LdNPV), and *Entomophaga maimaiga* fungi. Bacterial pathogens in addition to *B.t.k.* and other *Bacillus* species that adversely affect the gypsy moth, include *Serratia marcescens, Serratia liquefaciens, Streptococcus,* and *Pseudomonas* spp. These microorganisms are associated with a collective mortality in the gypsy moth of less than or equal to 15% (Podgwaite 1981). The gypsy moth nucleopolyhedrosis virus (LdNPV) is a natural component of the gypsy moth environment (Podgwaite 1979; 1981; Podgwaite and Campbell 1970; Lindroth et al. 1999) and is considered the primary natural regulator of dense gypsy moth populations in North American forests (Glaser and Chapman 1913; Doane 1970). High density populations of gypsy moth will eventually collapse, for the most part due to pathogens, especially NPV (Elkinton and Liebhold 1990). *B.t.k.* and LdNPV are also control agents for the gypsy moth, and are addressed individually in separate risk assessments.

In addition to viral and bacterial pathogens, several fungal pathogens will infect gypsy moth populations, including species of *Paecilomyces*, *Fusarium* and *Verticillium* (Hajek 1997). Most fungal pathogens, however, appear to account for insignificant levels of recorded gypsy moth mortality (Podgwaite 1981). A major exception, however, is *Entomophaga maimaiga*, which plays an important role in gypsy moth population dynamics on other continents and which is widely established in North America. Nealis et al. (1999) estimate that *E. maimaiga* may account for approximately 4-14% of mortality in gypsy moth larvae. Infections with *E. maimaiga* tend to be more prevalent than naturally occurring infections from NPV in areas with low egg mass density (Buss et al. 1999). In low density plots, *E. maimaiga* increased mortality substantially only in 5th instar and later instars. In high density plots, earlier instars were also infected (Hajek 1997; Hajek et al. 2001). Models for the influence of *E. maimaiga* on gypsy moth populations have been developed by Weseloh (1998a, 1999, 2002, 2003).

The gypsy moth is at risk of significant predation by mammals, birds, and other insects. In general, invertebrates are the major predators of gypsy moth larvae, while small mammals are the major predators of pupae (Grushecky et al. 1998). Mice and shrews are important predators of gypsy moth, particularly during the pupal stage (Bess et al 1947; Jones et al. 1998) or when the population density of the gypsy moth is low (Elkinton et al. 1996, 2002). When the population density of small mammals is high, small mammals may be a major source of predation on larvae (Cook et al. 1995). When populations of small mammals are low, the relative importance of predation by terrestrial invertebrates increases (Hastings et al. 2002a,b).

Forbush and Fernald (1896) first identified birds as predators of gypsy moth larvae. Some species of birds even prey on egg masses (Cooper and Smith 1995). In general, however, mammals appear to have a greater impact on gypsy moth populations than birds (Smith and Lautenschlager 1981; Elkinton and Liebhold 1990).

Numerous insects act as parasites or predators to the gypsy moth, including the larvae of various tachinid flies and braconid wasps (Hajek 1997). Extensive efforts were made to introduce European and Asian gypsy moth parasitoids to North America (parasitoids are insects, especially flies and wasps, that complete their larval development inside the body of another insect). Ten species have become established (Elkinton and Liebhold 1990). Gypsy moth mortality due to each type of parasite is specific to a given gypsy moth life stage. The venom of the ectoparasitic wasp *Microbracon hebetor*, contains a toxin that inhibits larval growth in gypsy moth (Masler and Kovaleva 1999). The food preference of certain wasps species—i.e., chalcids—seems to depend on the sex of the pupae (Fuester and Taylor 1996). Although invertebrate predation of gypsy moth pupae may be minor compared with vertebrate predation (Campbell and Sloan 1977a), Smith and Lautenschlager (1981) suggest that mortality attributed to vertebrates may be caused by invertebrates, like ground beetles (Elkinton and Liebhold 1990). Both adult and immature stages of *Calosoma sycophanta*, a large ground beetle introduced from Europe, are known to feed on gypsy moth larvae and pupae (Elkinton and Liebhold 1990). In addition, Weseloh (1996b, 1998b) suggest that predation by ants, particularly on gypsy moth larvae that fall to the forest floor, could cause significant mortality to gypsy moth larvae.

3. HUMAN RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION
3.1.1. Overview
Skin irritation after contact with larvae of many species of lepidoptera is common and this effect is the most common and best documented response to contact with gypsy moth larvae. The skin reactions seem to be associated with contact with small fine hairs that stick out from the body of the larva. The precise mechanism or mechanisms of action for these irritant effects is unclear but may involve three general responses: mechanical irritation, toxic reaction to a compound such as histamine, and an immediate or delayed allergic reaction. Raised and reddened areas of skin, known as wheals, are the most characteristic skin lesions. These lesions, resembling the raised patches of skin often associated with mosquito bites, may be approximately 0.25–0.5 inches in diameter. Contact with larvae may also cause rashes rather than wheals. Both wheals and rashes may cause severe itching that can persist for several days to 2 weeks and may be sufficiently severe to cause the affected individual to seek medical treatment. Other effects that may be associated with exposure to gypsy moth larvae include eye and respiratory irritation but these effects are less well documented, compared with dermal effects.

In very severe infestations, the large numbers of larvae in an area may cause stress or anxiety in some individuals. Also during heavy infestations, water quality may be affected by increased runoff and by direct contamination with frass. Nonetheless, there are no documented cases of changes in water quality being associated with adverse effects in humans.

3.1.2. Mechanisms of Action
As discussed in Section 3.1.3, dermal irritation is the most common adverse effect associated with human exposure to gypsy moth larvae. Dermal reactions to contact with lepidopteran larvae are in general relatively common (Anonymous 1984; Gilmer 1925; Goldman et al. 1960; Hellier and Warin 1967; Katzenellenbogen 1955; Perlman 1965; Schmidt 1982; Wirtz 1980,1984). Moreover, the gypsy moth is the most common insect associated with allergies—i.e., 28.7% of known cases (Wirtz 1980).

The skin reactions seem to be associated with contact with the larval setae, small fine hair-like protrusions from the body of the larvae (Allen et al. 1991). The precise mechanism or mechanisms of action for these irritant effects is unclear but may involve three general responses: mechanical irritation, toxic reaction to a compound such as histamine, and an immediate or delayed allergic reaction (Burnett et al. 1989, Shama et al. 1982). Gypsy moth larvae have four kinds of setae, two of which are hollow and attached to glandular cells. The hollow setae are suspect, but not unequivocally identified as the setae associated with skin reactions in humans (Anderson and Furniss 1983). According to several case reports and epidemiology studies, dermal effects in humans are usually associated with exposure to the first instars (Anderson and Furniss;1983, Tuthill et al. 1984). Whole first instars and the setae of fifth instars contain histamine (Shama et al. 1982), a compound that causes wheals, which are characteristic of dermal contact with gypsy moth larvae (Sullivan 1982).

The study by Beaucher and Farnham (1982) supports the association between gypsy moth exposure and allergic responses. In the study, closed patch tests were conducted on 8 individuals who had a history of skin reactions to the gypsy moth and 11 individuals, with no such history, who served as controls. A positive response to the patch test was observed in each of the individuals who had a history of skin reactions to the gypsy moth and in only one individual in the control group. The observed response was consistent with the reported dermal effects of gypsy moth exposure. In some cases, severe itching (pruritis) kept individuals awake at night. In general, the time from exposure to the onset of the reaction was 24–48 hours, suggesting a delayed hypersensitivity similar to poison ivy reactions. In another study, 10 of 17 workers at a laboratory conducting research on the gypsy moth reported a history of adverse skin or respiratory reactions. According to the results of scratch tests, 7 of the 10 workers who reported a history of adverse reactions were allergic to gypsy moth parts or other gypsy moth substances. The intensity of the response, based on a categorical classification of skin responses, was greater for extracts of cast larval skins and whole larvae than for egg mass hairs (Etkind et al. 1982).

3.1.3. Effects on Skin

Reports of dermal responses to contact with gypsy moth larvae began with the introduction of the moth to the United States. A late 19th century document describes a situation in which an individual in Medford Massachusetts *"... was poisoned by them [gypsy moths]. While killing them upon the trees they would get upon his neck and blister and poison it"* (Forbush and Fernald 1896, p. 16). A few years later, a physician in Boston reported a number of cases of *"...inflammation of the skin, which were undoubtedly caused by contact with some caterpillar ... which must be some recently introduced species"* (White 1901 p. 599). Although Dr. White attributed these cases to the brown-tailed moth (*Euproctis chrysorrhea*), they are consistent with the reported effects of exposure to the gypsy moth which had escaped into the area near Boston some years before (Section 2.2). The literature contains no further mention of human health effects associated with the gypsy moth for almost a century.

In the early 1980s, there was a massive gypsy moth infestation in the northeastern part of the United States. In 1981, outbreaks of itchy skin rashes that coincided with the heavy infestations were widespread and a source of public annoyance (Marshall 1981). Coincident with this infestation, reports describing the human health effects associated with exposure to the gypsy moth appeared in the medical literature.

Wheals, raised and reddened areas of skin, are the most characteristic skin lesions associated with human contact with the larvae. These lesions, resembling the raised patches of skin often associated with mosquito bites, may be approximately 0.25–0.5 inches in diameter and are surrounded by an area of redness. In severe cases, the wheals may be so numerous that they overlap on large areas of the skin, a condition referred to as urticaria. Contact with the larvae is reported also to cause contact dermatitis, characterized by a rash rather than wheals (Anderson and Furniss 1983). Both rashes and wheals may cause severe itching, also known as pruritis. This effect can persist for several days to 2 weeks and may be sufficiently severe to cause the the

affected individual to seek medical treatment (Aber et al. 1982; Allen et al. 1991; Shama et al. 1982).

During the severe infestations in the early 1980s, there were three published reports regarding the development of skin reactions in school children (Aber et al. 1982; Anderson and Furniss 1983; Tuthill et al. 1984). In the spring of 1982, a telephone survey was conducted to collect information from approximately 1000 people (representing more than 90% of those selected for study) in one highly infested community (HI, Lunenburg) and one minimally infested community (LO, Medway) in Massachusetts (Tuthill et al. 1984). The risk of developing a dermal response over a 1-week period was 10.4% in the HI community and 1.6% in the LO community. The responses occurred most often in individuals who had developed rashes during the previous year or who had direct contact with the larvae (that is, larvae crawled on them). The combination of these two factors resulted in an additive increase in risk. Other variables related to increased response included a history of hay fever and the practice of hanging clothes outdoors to dry. The rates at which the dermal responses developed in individuals in the HI community were inversely associated with age (18.8% in 0- to 12-year olds, 10.2% in 13- to 59-year olds, and 2.1% in 60-year olds and older individuals). The average prevalence of dermal responses in both communities combined, 1 week before the emergence of the first instars, was 1.3% (Tuthill et al. 1984).

Sometime between the end of April and the third week of May, 1981, there was an increased incidence of rashes among students in two schools in Northeast Pennsylvania (Aber et al. 1982). School A had a response rate of 42.2% (135 of 320 students), and school B had a response rate of 25.3% (76 of 300 students). The dermal responses included pruritic rash and occasional urticaria, usually located on exposed areas of the body. Based on the results of a survey of students from the same schools who were not affected by the gypsy moth, the investigators determined that there was a statistical association between touching larvae (p<0.01), working in a garden (p<0.05), or going fishing (p<0.01) and the incidence of rashes.

Concurrent with the infestation in Pennsylvania was an infestation in Connecticut, associated with an outbreak of skin reactions in students at several schools within the community (Anderson and Furniss 1983). Urticaria was observed in 7.2% of the 2600 students attending four schools in Newton, Connecticut. More than 50% of the cases of urticaria occurred during the first week in May, coinciding with the emergence of first instars. Very few cases (approximately 10) occurred during the third week of May when the larvae were predominantly in the third instar stage. In Burlington, Connecticut, the incidence of skin reactions was approximately 5.1% (96 of 1870). In another school, about 7.1% (75 of 1058) of students were affected. In Bristol, Connecticut, there were 1348 cases of rashes in the public schools, amounting to approximately 10.7% of the total student enrollment (12,500). Health officials estimated that the true prevalence may have been 3 times higher than reported; however, details supporting this assessment were not provided. Nonetheless, the estimate is consistent with the occurrence of rashes in 12 of 25 children attending a nursery school in the same community.

3.1.4. Effects on Eyes and Respiratory Tract
The ocular and respiratory effects in humans after exposure to the gypsy moth or other lepidopteran larvae are less well documented, compared with dermal effects. Of the 10 workers with a history of adverse reactions to the gypsy moth (Etkind et al. 1982), all 10 had skin reactions, 4 had eye irritation, and 2 had respiratory reactions. In a survey of laboratories conducting research on insects, 28.7% of all reported allergies were attributed to the gypsy moth. The most frequent reactions among affected individuals were skin irritation (61%), sneezing or runny nose (67%), and eye irritation (60.9%). Labored respiration was observed in 33% of the affected individuals (Wirtz 1980). The frequencies of these reactions are for all individuals who had adverse health effects after exposure to insects in general, not just the gypsy moth. In the early 1980s, NIOSH conducted a survey of workers in USDA/ARS research facilities who were involved in rearing insects for various research projects. As in the study by Wirtz (1980), the most common respiratory or ocular symptoms included sneezing/runny nose (73%), ocular irritation (68%), cough (38%), wheezing (26%), and shortness of breath (24%) (Anonymous 1984). An update of this survey was planned for the early 1990 (Petsonk 1994) but no such publication was found in the literature.

The severity of ocular or respiratory effects in humans after exposure to the gypsy moth is not well characterized; however, these effects appear to be reversible. Although some respiratory effects may involve pain, there are no data to indicate that the respiratory effects are life threatening or require hospitalization (Perlman 1965; Shama et al. 1982).

3.1.5. Other Potential Effects
The stress or anxiety associated with gypsy moth infestations is difficult to assess. This stress has not been associated with frank health effects. In many communities, the stress may be exacerbated by disputes about appropriate approaches for dealing with the pest (Williams 1982). Anecdotal reports suggest that some people may be extremely anxious about infestations (National Gypsy Moth Management Group 1991, p. 3):

> *... the mere mention of insects sends some people into fits of scratching, but phobia was not an adequate explanation for the epidemic of runny noses, irritated eyes, and rashes that happened to coincide with the occurrence of gypsy moth caterpillars last spring [1990]. Every [Pennsylvania] county and state gypsy moth office received numerous calls and one agency was reported to have received over 2,700.*

Moreover, reports regarding the willingness of populations to pay for gypsy moth control (Miller and Lindsay 1993a,b) suggest that gypsy moth infestations are regarded as highly undesirable by the general public, both in terms of aesthetic damage and the potential for adverse effects on human health. Among 629 individuals residing in infested areas, the most frequent reasons for a willingness to pay for control measures against the gypsy moth were aesthetic damage (15%) and

the nuisance factor (13%) (Miller and Lindsay 1993b). Concerns regarding adverse health effects directly related to exposure were expressed by 4% of the responders.

In most instances, gypsy moth defoliation will have little effect on adjacent water bodies (Corbett and Lynch 1987; Grace 1986). During heavy infestations, water quality may be affected by increased runoff and by direct contamination with frass. During active defoliation, fecal streptococci levels in stream water were as high as 25,000/100 mL and fecal coliform densities exceeded 90/100 mL (Corbett 1991). Long term studies of the impact of gypsy moth defoliation on water quality have included studies that show stream water chemical concentrations following defoliation that have included increasing amounts of strong acid anions, base cations and hydrogen ions, as well as decreasing concentrations of acid neutralization capacity (Webb et al. 1995). In addition dissolved nitrogen as nitrate will increase in streams following gypsy moth defoliation (Eshleman et al. 1998).

There are neither studies that directly address the contamination of water with frass nor reports in the literature of adverse effects on human health associated with water contamination from frass. In gypsy moth defoliated forests, however, frass output reached 756 kg (dry weight)/ha in a 1 month period (Grace 1986).

Lyme disease, which is a bacterial infection induced by *Borrelia burgdorferi*, causes serious health effects in humans. In the northeastern and central United States, the primary vector is the black-legged tick, *Ixodes scapularis* (CDC 2004). The tick can infect the white-footed mouse, *Peromyscus leucopus*, and ticks from the white-footed mouse can infect deer or humans (Ostfeld 2002; Ostfeld et al. 1996). As discussed in Section 4.1.2.1, the white-footed mouse eats acorns produced by oak trees. Gypsy moth infestations or outbreaks may result in decreases in acorn production due to damage to oak trees, which, in turn, may cause decreases in the population of white-footed mice due to decreases in food abundance (Elkinton et al. 1996, 2002). There is speculation that this decrease in the population of mice may limit the transmission of Lyme disease to humans due to the adverse effect on the primary vector (Jones et al. 1998; Randolph 1998). Currently, however, there does not appear to be sufficient information to assess the plausibility of this supposition.

3.2.   EXPOSURE ASSESSMENT
3.2.1. Overview.
The number of larvae per unit area or tree might be considered the most direct and relevant measure of human exposure because it is contact with the larvae that causes skin irritation, the adverse effect typically associated with the gypsy moth. The available dose response data, however, are based on studies in which exposure is quantified as the number of eggs masses per acre and thus this is the exposure measure that is used in this risk assessment. As long as gypsy moth populations remain sparse, the larvae usually eat only a small proportion of the foliage of even their most favored host species, and contacts with people are rare.   In such cases, egg masses generally do not exceed 50 egg masses/acre. During full-scale outbreaks, densities of about 5000 egg masses/acre are common and densities greater than 20,000 egg masses/acre are occasionally recorded.

3.2.2. Exposure Metameter.
Gypsy moth populations can be monitored by estimating the numbers of egg masses (typically expressed as egg masses per acre), the number of larvae (which can be expressed as larvae or larval mass per unit area or larvae per tree), or the number of adults per unit area. For adult moths, population surveys usually involve the use of pheromone traps with or without an insecticide. Surveys of larval populations may involve band trapping, direct examination, or correlations between frass volume and population density. Measurements of larval populations can be highly variable over time and among different species of trees. For example, Naidoo and Lechowicz (2001) conducted larval counts on different species of trees in a deciduous forest in Quebec. In preferred tree species (i.e., red oak), larval populations were as high as 250 larvae per tree. In less preferred tree species, larval populations were much lower, ranging from about 4 larvae per tree (white ash) to 10 larvae per tree (sugar maple). In terms of larval mass, values of 8.4 kg/ha in the month of June and 16 kg/ha in the month of July were measured during severe infestations (Grace 1986).

While the number of larvae per unit area or tree might be considered the most direct and relevant measure of human exposure, the available dose response data (Section 3.3) are based on studies in which exposure is quantified as the number of eggs masses per acre (i.e., Tuthill et al. 1984; O'Dell 1994). As long as gypsy moth populations remain sparse, the larvae usually eat only a small proportion of the foliage of even their most favored host species, and contacts with people are rare. In such cases, egg mass densities generally do not exceed 50 egg masses/acre.

For several years, gypsy moth populations may exist in a density range high enough (between 50 and 500 egg masses/acre) to make the insect a minor nuisance in wooded communities and cause partial defoliation. Once, however, the gypsy moth population increases to a full-scale outbreak, the combination of insect frass and leaf fragments, loss of shade at midsummer, and the large number of larvae may become a major nuisance (Williams 1982). Although the duration of such outbreaks is unpredictable, the principal factors that influence the pest include a variety of pathogens, intraspecific competition for food, and inclement weather (Campbell 1981; Podgwaite

1981; Miller et al. 1989). During full-scale outbreaks, densities of about 5000 egg masses/acre are common and densities greater than 20,000 egg masses/acre are occasionally recorded.

Egg mass densities in infested areas tend to be lower in areas where the human population is dense, compared with less densely populated areas. At the forest periphery, however, egg mass densities can be much higher and seem to be associated with man-made objects (Campbell et al. 1976). Within a relatively limited geographical range, egg mass densities may vary remarkably. For instance, in a heavily infested area with a mean egg mass density of approximately 3800 egg masses/acre, egg mass counts ranged from 0 egg masses/0.1 acre surveyed to 1000 egg masses/0.1 acre surveyed (O'Dell 1994). Similar variability in egg mass density were observed in larger survey areas, as well (Reardon et al. 1993). During a heavy infestation, as many as 50,000 larvae may inhabit a single tree. At such extremely dense concentrations, the generation of frass may be sufficiently intense to be audible, sounding like a light rain (Beaucher and Farnham 1982).

3.2.3. Intensity of Exposures
Given the localized variability in larval populations, quantitative estimates of exposure to larvae cannot be made. Epidemiology studies conducted in gypsy moth infested communities suggest that larval density as a measure of the intensity may not be meaningful. The most important factor in assessing exposure may be the probability of coming into contact with one or more larvae, rather than the number of larvae in a population. In this respect, patterns of human behavior, such as the amount of time spent outdoors and certain kinds of activities likely to result in contact with larvae may be more important than measurements of the local larval population. The likelihood of human exposure to the gypsy moth is likely to increase in proportion to the increases in the larval population in a given area; however, it is not possible to estimate more precise relationships of larval population density to human exposure.

3.3. DOSE-RESPONSE ASSESSMENT
3.3.1. Overview
The dose-response assessment for human health effects is based on reports of skin irritation in two populations: one with low exposure (an average of 32 egg masses/acre) and the other with high exposure (an average of 3809 egg masses/acre) (Tuthill et al. 1984). The low-exposure group exhibited no increase in skin irritation and 32 egg masses/acre is taken as a NOAEL for humans and is used as a surrogate RfD for exposure to the gypsy moth in a manner analogous to the use of RfD values for control agents. The high exposure group did evidence a significant increase in skin irritation. Based on the observed dose-response relationship, egg mass densities up to 128 egg masses/acre are not likely to cause a detectable increase in skin irritation or rashes. In addition to these quantitative estimates, the severity of the response is important, particularly in a comparison of effects caused by exposure to the gypsy moth and effects caused by exposure to the agents used to control the gypsy moth. Dermal responses to the gypsy moth are sufficiently severe to have generated numerous case reports. While precise statistics are not available, it does appear that the severity of the skin irritation is sufficient to cause appreciable numbers of affected individuals to seek medical care. While exposure to the gypsy moth is associated with irritation to the eyes and respiratory tract, quantitative dose-response relationships for these endpoints cannot be developed.

3.3.2. Effects on Skin
Of the several available studies that demonstrate skin irritation in humans after exposure to gypsy moth larvae (see Section 3.1.3), the study by Tuthill et al. (1984) is the most appropriate from which to derive a quantitative dose-response assessment. Tuthill et al. (1984) investigated adverse dermal responses in many individuals after exposure to the gypsy moth in areas of high and low infestation. As summarized in Table 3-1, the most relevant data are taken from two groups, one that consisted of 557 individuals in an area of low infestation (Medway, Massachusetts) and one that consisted of 508 individuals in an area of high infestation (Lunenberg, Massachusetts). Although the survey was conducted in the summer of 1982 over two time periods, prior to and after larval emergence, the exposure estimates are based on egg mass counts taken in the fall of 1981. In the Tuthill et al. (1984) publication, the egg mass counts are given only as ranges: 10 to 10,000 egg masses/acre in the area of high infestation and 0 to 70 egg masses /acre in the area of low infestation.

One of the coauthors of the Tuthill et al. (1984) study surveyed egg masses in the two communities (O'Dell 1994). In the high exposure community, surveys were conducted on 27 0.1-acre plots throughout the infested area between October 5 and 6, 1981. In the high exposure areas (Lunenberg), the average egg mass density was 3809 egg masses/acre. In the low exposure community (Medway), 20 sites were surveyed. The arithmetic average number of egg masses/acre was 32, but the egg masses were unevenly dispersed. No egg masses were found at 15 of the 20 sites, and egg mass counts at the other 5 sites were 2, 2, 3, 7, and 50. These egg mass counts were made in the fall, before the outbreak of rashes in the following summer. The use of these egg mass densities as a surrogate for estimating exposure to larvae is based on the assumption that there is a positive correlation between the number of viable larvae in the summer

and the number of egg masses in the preceding fall. Occasionally, below normal midwinter temperatures have resulted in high mortality among overwintering eggs (Bess 1961). Usually, however, fall egg mass counts are closely related to subsequent larval density, particularly among early instars. For the dose response assessment, the average egg mass counts are used for each site—i.e., 32 egg masses/acre for Medway and 3809 egg masses/acre for Lunenberg.

In the Tuthill et al. (1984) study, response data for both the low and high exposure areas are presented as the number of individuals with and without signs of dermal irritation. This type of data is typically termed quantal or discrete and can be used to assess the statistical significance of differences between two groups using the Fisher Exact Test (Uitenbroek 1997). Typically, the Fisher Exact Test is used to determine if there are significant differences between a control group and an exposed group. The Fisher Exact Test yields a p-value, the probability that the observed difference occurred by random chance. If the p-value is very low, the differences are considered statistically significant. Typically, a p-value of 0.05 is used as the maximum value for asserting that the differences are significant. If the p-value is greater than 0.05, the differences are not regarded as statistically significant.

The Tuthill et al. (1984) study does not include an actual control group—i.e., a population in an area where no gypsy moth were present; however, for both Lunenberg and Medway, the investigators provide responses before and after larval emergence. Consequently, within each group, the response rate prior to larval emergence can be considered a "control" response and the response rate after emergence can be considered a response associated with exposure to the gypsy moth larvae. Using the Fisher Exact Test, the Medway population demonstrates no statistically significant response after larval emergence. In other words, the p-value is 0.3 for the comparison of response rates before and after exposure and the probability that this difference could be due to random variation is 0.3 or 30%. Thus, the exposure estimate of 32 egg masses/acre may be considered a NOAEL (no-adverse effect level). For the Lunenberg population, however, the post-emergence response rate of 50/508 is significantly higher than the pre-emergence response rate of 7/508 and the p-value is 8×10^{-10}. In other words, the probability that the difference is due to random variation is only 8 in 100 million. Thus, the exposure estimate of 3809 egg masses/acre may be considered a LOAEL (lowest observed adverse effect level).

In addition to the pre- and post-emergence dermatological response rates for all individuals in the two areas, Tuthill et al. (1984) also provide post-emergence data on three different age groups: 0-12 years, 13-59 years, and >59 years. Because no pre-emergence response data are provided by age group, no statistical analysis on "control" vs exposed groups can be conducted. Nonetheless, within the high exposure groups (Lunenberg), an age-response pattern is clearly apparent with younger individuals being much more sensitive than older individuals. As indicated in Table 3-1, these differences are both statistically significant and substantial, spanning a nearly 10 fold difference in sensitivity—i.e., 2.1% in older individuals vs. nearly 20% in individuals in the 0-12 year age range.

Whether or not these different response rates in the different age groups represents a true age-specific difference in sensitivity is unclear. Young children, compared with adults, are likely to spend more time out of doors and may be more likely to come into contact with gypsy moth larvae. As indicated in Table 3-1, there is a clear association between the number of individuals who reported touching larvae and the number of individuals who developed a rash after touching larvae. Thus, it is plausible that the age-specific pattern apparent in the data from Tuthill et al. (1984) could be an artifact of greater contact with gypsy moth larvae by younger individuals.

Because Tuthill et al. (1984) included only two exposure groups and no true control group, a more quantitative dose-response assessment is limited. The U.S. EPA (2001) developed a series of models for estimating benchmark doses. As defined by U.S. EPA (2001), the benchmark dose is the estimate of the lower range of a confidence interval for a dose or exposure associated with a defined response rate. For example, a benchmark dose could be calculated as the 95% lower limit for an exposure associated with a 10% response.

Using the U.S. EPA (2001) benchmark dose software, benchmark doses for both 1% and 10% responses were calculated for all groups combined as well as for each age-group. Because of the limited exposure data, the simple exponential model is used:

$$P = 1 - \exp(\beta * EM)$$

where β is the potency parameter in units of proportion responding per egg mass/acre and EM is the number of egg masses/acre. These analyses are summarized in Table 3-1. For all groups combined, the pre-exposure responses (Table 3-1) were used as zero exposure or control responses. For the age-group specific modeling, no control group was used—i.e., the model has zero degrees of freedom. Thus, the *p*-values shown in Table 3-1 are just an indication of whether or not the potency parameter was significantly different from zero.

The results of the dose response modeling are qualitatively consistent with the use of the simpler Fisher Exact Test. The potency parameter is greatest in the 0- to12- year-old groups. The dose-response relationship for the >59-year-old group is not statistically significant (*p*=0.15), indicating no substantial response in individuals more than 59 years old in either Lunenberg or Medway. Based on the most sensitive individuals, egg mass densities of 128 egg masses/acre are likely to cause adverse effects in no more than 1%—i.e., a response rate unlikely to be detectable in an epidemiology study. Egg masses of 1336, however, are likely to cause a response rate of at least 10%, which would be detectable in a well-conducted epidemiology study. Again, these results are essentially consistent with the NOAEL and LOAEL values discussed above.

For the current risk assessment, the NOAEL of 32 egg masses/acre is used as a surrogate RfD for exposure to the gypsy moth in a manner analogous to the use of RfD values for control agents. While an uncertainty factor is typically applied to NOAEL values to estimate an RfD, no uncertainty factor is used for this risk assessment. This approach seems reasonable based on the

benchmark dose modeling which indicates that egg mass densities up to 128 egg masses/acre are not likely to cause a detectable increase in skin irritation or rashes.

In addition to these quantitative estimates, the severity of the response is important, particularly in a comparison of effects caused by exposure to the gypsy moth and effects caused by exposure to the agents used to control the gypsy moth. Dermal responses to the gypsy moth are sufficiently severe to have generated numerous case reports as well as a study by NIOSH (see Section 3.1.3). One of the criteria for judging the severity of any response is whether or not an individual will seek medical attention as the result of an exposure to a particular agent. No precise statistics on seeking medical attention after exposure to gypsy moth larvae are available. Tuthill et al. (1984) have noted that: *"Less than 10 per cent of the sufferers sought medical care."* As discussed further in the risk comparison for these agents, a response rate of 10% is substantially greater than rates for any of the agents used to control the gypsy moth, based on comparable data.

3.3.3. Other Effects

As discussed in Section 3.1, exposure to the gypsy moth is associated with irritation to the eyes and respiratory tract as well as generalized psychological distress during severe infestations. No data, however, are available for developing quantitative dose-response relationships for these endpoints.

3.4. RISK CHARACTERIZATION
3.4.1. Overview
In sparse to moderate infestations—i.e., egg mass densities of <500 egg masses/acre—adverse effects involving skin irritation are not likely to be detectable in populations of exposed humans. Nonetheless, some individuals who come into contact with gypsy moth larvae could develop skin irritation. In heavy gypsy moth infestations—i.e., >500 to 5000 egg masses/acre—adverse skin reactions would be expected in substantial numbers and the effects would likely be sufficiently severe to cause some individuals to seek medical attention. In extreme outbreaks—i.e., >5,000 to 20,000 egg masses/acre— the effects will be qualitatively similar to those of severe infestations but could affect up to about one-third of the population. Heavy infestations or extreme outbreaks could cause ocular and respiratory effects in some people but the likelihood of observing these effects cannot be quantified. Similarly, severe infestations are often considered to be a nuisance and cause aesthetic damage to the environment. Both of these factors can lead to stress in some individuals. There is speculation that severe damage to oak forests from gypsy moth infestations might result in a decrease in the prevalence of Lyme disease. This effect of gypsy moth exposure obviously would be viewed as beneficial to human health. Currently, however, there does not appear to be sufficient information to assess the plausibility of this supposition. Young children may be a group at special risk from effects of gypsy moth exposure but it is not clear whether children are more sensitive than adults to the effects of gypsy moth exposure or whether responses in children appear greater because children spend more time outdoors compared with adults.

3.4.2. Effects on Skin
The likelihood of adverse skin reactions in humans after exposure to gypsy moth larvae can be quantified at least in terms of egg mass density (see Section 3.3). Skin irritation also may be considered the most sensitive effect. That is, if exposure levels are less than levels at which a substantial increase in skin irritation is observed, other effects are not likely to be seen.

The risk characterization for the general public is summarized in Table 3-3. The ranges of risk for the general public are based on the ranges of exposure given in column 2 of this table. As in the ecological risk assessment, the stratification of sparse to extreme infestations in terms of eggs masses/acre is somewhat arbitrary but covers a sufficiently broad range to encompass most egg mass densities that are likely to be encountered —i.e., from 50 to 20,000 egg masses/acre. Egg mass densities of 50 egg masses/acre or less are characteristic of mild infestations that occur in the south central region of the United States (Davidson et al. 2001). Egg mass densities of 20,000 egg masses/acre or more are uncommon but can occur in localized areas during gypsy moth outbreaks (Hajek 1997).

Three types of risk characterizations are provided in Table 3-3. The first is based on the NOAEL of 32 egg masses/acre. As discussed in Section 3-3, this value is used as a surrogate RfD for exposure to the gypsy moth. As with all hazard quotients (HQs) based on an RfD, an HQ of less than one indicates that no adverse effects are plausible. The second type of risk characterization is based on a LOAEL of 1336 egg masses/acre. As indicated in Table 3-2, this value is the

estimated benchmark dose associated with a 10% response in the most sensitive subgroup (children < 13 years old). This value is considered a LOAEL rather than a NOAEL because a response rate of 10% would be detected in an epidemiology study and because the value is very close to the observed LOAEL of 3809 egg masses/acre in the study by Tuthill et al. (1984). The interpretation of the hazard quotients based on this LOAEL is different from standard hazard quotients based on an NOAEL or RfD—i.e.,values greater than 1 indicate that adverse effects are likely to be observed in the exposed population.

In addition to the risk characterizations based on the NOAEL/RfD and LOAEL, the last column in Table 3-3 gives the upper range of extra risk associated with each of the exposure categories. These values are derived from the U.S. EPA (2001) benchmark dose software using the one-hit model, as discussed in Section 3.3.

Taken together, all three numerical expressions of risk lead to a consistent qualitative risk characterization. In sparse to moderate infestations—i.e., egg mass densities of <500 egg masses/acre—adverse effects involving skin irritation are not likely to be detectable in populations of exposed humans. Nonetheless, some individuals who come into contact with gypsy moth larvae could develop skin irritation. In heavy gypsy moth infestations, defined in Table 3-3 as ranging from >500 to 5000 egg masses/acre, it is likely that adverse skin reactions will be reported and that the effects will be sufficiently severe to cause some individuals to seek medical attention. In extreme outbreaks—i.e., >5,000 to 20,000 egg masses/acre— the effects will be qualitatively similar to those of severe infestations but could affect up to about one-third of the population.

3.4.3. Other Endpoints
As discussed in the hazard identification (see section 3.2), exposure to gypsy moth larvae is associated with ocular and respiratory effects in humans. In addition, infestations are often considered to be a nuisance and cause aesthetic damage to the environment. Both of these factors can lead to stress in some individuals. Also during severe infestations, water quality may be affected. While all of these concerns may be qualitatively associated with exposure to the gypsy moth and while the severity of these effects are likely to increase with the increasing severity of gypsy moth infestations, no quantitative dose-response assessment can be made (see Section 3.3.3). Accordingly, no quantitative risk characterization can be provided.

As discussed in Section 3.1.5, there is reason to speculate that severe damage to oak forests could result in a decrease in the prevalence of Lyme disease. This effect of gypsy moth exposure obviously would be viewed as beneficial to human health. Currently, however, there does not appear to be sufficient information to assess the plausibility of this supposition.

3.4.4. Sensitive Subgroups
Young children may be a group at special risk from effects of gypsy moth exposure. Although this is suggested in the study by Tuthill et al. (1984) as well as by studies on school children affected by gypsy moth infestations (Aber et al. 1982; Anderson and Furniss 1983), it is not clear

whether the finding indicates that children are inherently more sensitive than adults to the effects of exposure or whether children have a greater incidence of response because they spend more time outdoors than adults and thus have great potential for exposure to gypsy moth larvae.

3.4.5. Connected Actions

There is no evidence to assess the consequences of connected actions involving the various program activities or other common activities associated with the gypsy moth. As discussed in the risk assessment on Gypchek, one of the agents used to control gypsy moths, Gypchek contains gypsy moth parts and may cause irritant effects similar to those caused by the gypsy moth. Consequently, it may be that the effect of simultaneous exposure to gypsy moth larvae and Gypchek would be additive.

3.4.6. Cumulative Effects

Two types of cumulative effects may be considered in assessing the consequences of exposure to the gypsy moth. During an infestation, repeated exposures will occur in the population for the duration over which exposure to the gypsy moth instars occurs. In addition, cumulative effects may be induced from year to year as infestations reoccur.   Cumulative effects from exposure to the larvae during a single season are essentially encompassed by the Tuthill et al. (1984) study, the epidemiology study on which the risk assessment is based, because the investigators monitored effects in populations during the period in which early instars were present. The available data do not permit a definitive assessment of the cumulative effects of exposure to the gypsy moth over several seasons. As discussed in the hazard identification (see Section 3.1.2), there is evidence to suggest that an allergic reaction may be one of the mechanisms involved in the dermal effects associated with exposure to the gypsy moth. Thus, it is plausible that some individuals may become sensitized to the gypsy moth after repeated exposures over 1 or more seasons.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION
4.1.1. Overview
The clearest primary effect of gypsy moth infestations is on terrestrial plants, primarily trees. Various instars of the gypsy moth larvae will feed on host trees and can cause extensive defoliation which can kill some of the infested trees. On a larger scale, the extensive defoliation and/or death of trees may result in secondary changes to vegetation, which will, in turn, affect other forms of vegetation as well as various animal species (primarily related to changes in habitat). Gypsy moth larvae appear to have definite food preferences; oak, birch, poplar and apple trees seem to be their favorite food sources. While both the European and Asian gypsy moth cause similar types of damage (i.e., defoliation), their feeding preferences are somewhat different with the Asian gypsy moth preferring a wider range of vegetation. Heavy defoliation is much more common among the oaks than among trees that are not particularly favored as food by the gypsy moth. For susceptible oaks, the effects of infestations on tree mortality varies according to the initial condition of the stand and the severity and frequency of defoliations. Generally, gypsy moth infestations result in mortality losses of less than 15% of total basal area. When heavy defoliation is followed by massive overstory mortality, existing shrub and herb cover increase dramatically due to increases in available light, moisture, and nutrients.

The only other group of organisms that are likely to be directly effected by the gypsy moth are some and probably very few other lepidopteran species, including the northern tiger swallowtail butterfly. The mechanisms for direct adverse effects may include bacterial contamination of the leaves by gypsy moth larvae and a decrease in the nutritional value of the leaves damaged by the gypsy moth. Most studies, however, do not indicate substantial direct effects on other insects, including lepidoptera. In some cases, increases may be seen in populations of insect predators of the gypsy moth.

There is no indication in the literature that the gypsy moth will cause direct adverse effects in most groups of animals. Indirect effects, associated with damage to vegetation, may be of substantial consequence to some species, including squirrels, mice, and other mammals that rely on acorns. Although some mammals consume insects, including the gypsy moth, there is no evidence that gypsy moth outbreaks have a substantial impact on insectivorous mammals. Similarly, there is little indication that birds or aquatic species will be adversely affected by the gypsy moth. In some species of birds, gypsy moth infestations and subsequent defoliation may be beneficial, especially for species of birds that favor dead wood as a habitat.

4.1.2. Terrestrial Organisms
4.1.2.1. Mammals – As discussed in Section 3.1 (Human Health Hazard Identification), direct exposure to gypsy moth larvae causes various irritant effects in humans —i.e., skin, eyes, and respiratory tract. In most species of mammalian wildlife, however, fur is likely to reduce the risk of direct contact between the gypsy moth and the skin of the animal, making skin irritation an

unlikely result of exposure. Evidence of irritation to the eyes and or respiratory tract in mammalian wildlife species after direct contact with the gypsy moth is not found in the literature.

Although the hazard identification for the direct effects of gypsy moth exposure in mammalian wildlife is basically negative, indirect effects may be of substantial consequence to some species, as discussed in Section 4.1.2.5. For instance, gypsy moth outbreaks that cause substantial defoliation and mortality in some tree species, particularly oaks, could adversely affect the production of acorns (Gottschalk 1990a; McConnell 1988), which may limit food availability for some forest mammals.

To determine the effects of a gypsy moth outbreak on a population of black bears (*Ursus americanus*), Vaughan and Kasbohm (1993) monitored the behavior of 54 radio-collared black bears in the Shenandoah National Park after a gypsy moth outbreak that caused widespread defoliation, hard mast (acorn) failures, and tree mortality. The outbreak had no apparent effects on cub production or mortality rates of cubs or adults. Although the bears exhibited different habitat preferences at all seasons, they did not avoid defoliated habitat. In the fall, before the gypsy moth infestation, the bears ate mostly acorns. When acorns were no longer available due to defoliation of oak trees by the gypsy moths , the bears switched to eating fruit, which had no apparent impact on the nutritional quality of their diets. Seventy-one percent of bear dens were in tree cavities, primarily in living oaks (mean diameter at breast height = 98 cm). Gypsy moth-induced mortality of den trees was high and by the end of the study, 54% of the living oaks used as dens were dead. While no short-term effects were noted, Vaughan and Kasbohm (1993) speculated that the long-term adverse impact of defoliation on black bears may be a reduction in den sites, with natural replacement possibly requiring 50 years. Conversely, black bears will use as dens the upturned stumps of large dead trees that have been blown over. These would be expected to increase after severe defoliation sufficient to cause tree mortality.

Variations in acorn and other mast production are directly related to variations in populations of squirrels, mice, and other small mammals (Brooks et al. 1998; Gorman and Roth 1989; Nixon et al. 1975). The size of the acorn crop in the fall directly affects the population density of mice living in oak-dominated forests the following spring (McShea and Rappole 1992; McShea and Schwede 1993). By damaging oak tress, gypsy moth infestations can decrease acorn production and a decrease in acorn production secondary to gypsy moth infestations has been shown to decrease the population of white-footed mice, *Peromyscus leucopus* (Elkinton et al. 1996, 2002).

Although some mammals consume insects, including the gypsy moth (see Section 2.4), there is no evidence that gypsy moth outbreaks have a substantial impact on insectivorous mammals. Also, there is no evidence that the effects of gypsy moth outbreaks on other insect populations will directly or indirectly affect mammals that prey on insects. Sample et al. (1996) found no significant effects on the consumption of insects by Virginia big-eared bats in areas of high gypsy moth infestation.

4.1.2.2. Birds – There is little indication that birds will be adversely affected by the gypsy moth. Based on predation by various species of birds on the gypsy moth compared to other hairless lepidoptera, some species of birds appear to avoid the gypsy moth as a prey species (Smith 1985). This suggests, at least indirectly, that the setae or hairs on the gypsy moth larvae may have irritant properties for birds. Direct adverse effects, however, have not been noted in the literature. Reported increases in nesting failures of various species of birds appear to be due to increased predation and/or increased weather stress, both associated with defoliation (Crocoll 1991; Thurber et al. 1994).

In some species of birds, gypsy moth infestations and subsequent defoliation may be beneficial, especially for species of birds that favor dead wood (snags) as a habitat. As a result of defoliation by the gypsy moth, the amount of dead wood increases, particularly in the upper story and the dense vegetation in lower forest strata, providing habitat that is scarce in closed-canopy forests. These secondary effects of gypsy moth outbreaks, which can be considered beneficial to numerous birds, are well documented in the gypsy moth literature (Bell and Whitmore 1997a,b**;** DeGraaf 1987; DeGraaf and Holland 1978; Showalter and Whitmore 2002). Bell and Whitmore (1997) report that available nesting and foraging resources increased for several bird species as result of more snags, windfall, and shrub cover after defoliation, while there was no substantial impact from upper canopy defoliation on birds residing primarily in the forest canopy. Only tree nesting and flycatching guilds appeared to be affected adversely by the moth infestation. Cavity-nesting birds also benefitted indirectly from a gypsy moth outbreak (Showalter and Whitmore 2002). Thurber (1993) noted that bird density increased in plots in which the defoliation was of low to moderate impact. Species richness increased from 19 to 23 species per plot, with declines noted only for tree nesters and flycatchers on high impact plots (Thurber 1993). Increases in low shrub and ground nesters, cavity nesters, low shrub and ground foragers, bark foragers, forest edge species, short-distance migrants, year-round residents, and woodpeckers were widespread, but most pronounced on moderate impact plots. DeGraaf and Holland (1978) reported similar results, finding significantly fewer numbers of only 4 out of 36 bird species examined in heavily defoliated areas. DeGraaf (1987) notes no substantial effects on abundance of various species of birds in defoliated and non-defoliated stands in central Pennsylvania studied over a two year period.

4.1.2.3. Reptiles and Amphibians (Predominantly Terrestrial) – There is very little information regarding the effects of gypsy moth infestations or outbreaks on amphibians or reptiles. In the short-term, gypsy moth defoliation could have a negative impact on some habitats occupied by reptiles and amphibians by increasing solar radiation on dead and down material, litter, and the other materials found above subterranean habitats; however, in the longer term, the defoliation-induced increases in dead and down material will be beneficial to reptiles and amphibians (Schweitzer 1988). Peterson (1990) conducted a field study in south central New York on the effect of gypsy moth infestations on the timber rattlesnake, *Crotalus horridus* and noted that gypsy moth-induced defoliation had an adverse effect on rattlesnakes, primarily through reductions in acorn production and the consequent decrease in the population of small rodents that the snakes eat (see Section 4.1.2.1).

4.1.2.4. Terrestrial Invertebrates – Some lepidopteran species may be adversely affected by gypsy moth outbreaks and at least some of these effects may be direct rather than secondary. Redman and Scriber (2000) examined the adverse effects of the gypsy moth on the northern tiger swallowtail butterfly, *Papilio canadensis*, and demonstrated several different mechanisms associated with the adverse effects. Direct effects included 100% mortality in *Papilio* larvae exposed to leaves painted with gypsy moth body fluids, and 84% mortality in *Papilio* larvae fed leaves from aspen stands infested with gypsy moth larvae. Although the cause of death in the *Papilio* larvae was not clear, the investigators speculate that it was generally due to bacterial contamination of the leaves by gypsy moth larvae, since sterilized leaves did not cause a significant increase in mortality. Moreover, the damage to aspen leaves caused by gypsy moth larvae decreased the nutritional value of the leaves, which led to reduced growth rate and survival of the *Papilio* larvae. In addition, fields studies conducted by Redman and Scriber (2000) demonstrated that proximity to gypsy moths increased the rate of parasitism of the *Papilio* larvae.

The potential adverse effects of gypsy moth outbreaks to lepidoptera was also investigated by Sample et al. (1996) in a study designed to compare lepidopteran populations in 50 acre plots in mixed oak, hickory, and pine forests in West Virginia. Contaminated plots were characterized as stands with average egg mass densities of 235-1156 egg masses/ha (95-468 egg masses/acre), larval abundance of about 68-111 larvae/50 g dry foliage, and defoliation rates up to 88% over a 3-year period. Uncontaminated plots were characterized as stands with average egg mass densities of about 15-180 egg masses/ha (6-72 egg masses/acre), larval abundance of about 4-18 larvae/50 g dry foliage, and no defoliation over a 2-year period with 40% defoliation in the third year. Decreases in abundance and richness of larvae and adults from the family Arctiidae (tiger moths) were apparent in plots infested with gypsy moth larvae, compared with uncontaminated plots. The differences were statistically significant for both abundance (p=0.038) and species richness (p=0.0015). No substantial differences were observed in other lepidoptera or other invertebrate taxa, although a significant increase was noted in braconid wasps. Sample et al. (1996) suggested that the increased abundance of braconids in the plots with gypsy infestation was likely due to increased host (i.e., gypsy moth) availability.

The study by Work and McCullough (2000) demonstrates further that the impact of the gypsy moth is negative to only a small proportion of the lepidopteran community, primarily species that feed on oak and for which the larval development of the affected species and gypsy moth presumably coincide. Although the study does not address the mechanism(s) by which the gypsy moths adversely affect the lepidopteran community, the investigators suggest they might include altered host/plant quality, increases in natural enemies, or microclimate changes. All but the latter mechanism are demonstrated in the study by Redman and Scriber (2000) discussed above. No significant effects were observed on generalist woody plant feeders. Summerville and Crist (2002) criticize the guild classification used by Work and McCullough (2000); however, it is not clear what impact the use of alternate guild classifications would have on the conclusions reached in the study.

Contrary to studies demonstrating the adverse effects of gypsy moth infestations to some macrolepidoptera, anecdotal reports suggest that certain lepidopteran species respond positively to gypsy moth infestations. Schweitzer (1988) claims that 1981 produced the highest number of butterfly species ever for the New Haven, Connecticut area, which for many years stood as the record for eastern North America, despite the record number of acres defoliated by the gypsy moth that same year.

4.1.2.5. Terrestrial Plants (Macrophytes)
4.1.2.5.1. Gross Effects on Trees – The clearest primary effect of gypsy moth infestations is on terrestrial macrophytes, primarily trees. Various instars of the gypsy moth larvae will feed on host trees and can cause extensive defoliation which can kill some of the infested trees. On a larger scale, the extensive defoliation and/or death of trees may result in secondary changes to vegetation, which will, in turn, affect other forms of vegetation with consequences to various animal species (primarily related to changes in habitat).

Trees that are defoliated by even 75% or more are likely to refoliate during the same season. The refoliated leaves are smaller and fewer, and repeated defoliations can cause additional reductions in leaf size (Wargo 1981a).   According to Wargo (1981b), trees that refoliate are completely out of phase with the season. Visually, for example, the condition of trees in a mixed composite stand of oaks (red, black, scarlet, and white) in eastern New England showed rapid decline in the year after defoliation and continued to decline slightly during the next 5 years. Following a single heavy defoliation, about 10 years passed before these trees returned to their predefoliation condition (Campbell and Sloan 1977b).

Parker (1981) identifies five key factors that determine the effects of tree defoliation. The factors include, severity (how much foliage is removed); frequency (the number of successive years of defoliation); timing (when in the growing season the tree is defoliated); pathogens (the presence and number of secondary organisms); and health and vigor (the physiological condition of the tree when it is defoliated). Defoliation appears to have a direct impact on root carbohydrates (Kosola et al. 2001, 2002). Most hardwood (or deciduous) trees are able to tolerate at least 2 years of defoliation before root starch content (useable energy) is depleted (Wargo 1981a).   Since most coniferous species store carbohydrate resources necessary to refoliate in the leaves, they are usually unable to survive a single, complete defoliation (Johnson and Lyon 1988). Further decline and possible death of previously defoliated eastern hardwood trees are due primarily to secondary organisms like the shoestring fungus, *Armillaria* species, and the twolined chestnut borer, *Agrilus bilineatus* (Wargo 1981b). The defoliator and borer cause adverse effects in the crown of the tree. The borer affects the main stem and the fungus attacks the roots (Wargo 1977, 1981b). Gottschalk (1994) notes that by removing weak, sickly trees from the forest population, fungus (*Armillaria* species) and tree borers (*Agrilus* species) play an important and positive role in forest health.

Previous stand disturbance, which may allow partial colonization of root systems by *Armillaria*, increases rhizomorph abundance (Twery et al. 1990; Wargo 1989). Even in the presence of

abundant rhizomorphs, however, non-defoliated and lightly defoliated trees may remain resilient (Twery et al. 1990). Stressed trees also provide an environment that favors the survival of the twolined chestnut borer (Twery 1991; Wargo 1977), which is attracted to volatile chemicals released by stressed oaks (Dunn et al. 1986a). The trees most susceptible to the pest are those with low stores of starch reserves; however, only the trees with extremely low winter root starch reserves are likely to die (Dunn et al. 1986b, 1987).

Factors that contribute to interspecies differences in response to defoliation include where in the tree reserve energy is stored, the amount of energy required to refoliate, and how much energy is needed to maintain growth during refoliation (Twery 1991). Hemlock, for example, usually will not survive even one complete defoliation (Stephens 1988), whereas some oaks on dry sites may survive repeated defoliations indefinitely (Houston and Valentine 1977; Bess et al. 1947; Twery 1991).

Heavy defoliation is much more common among the oaks than among trees that are not particularly favored as food by the gypsy moth. For susceptible oaks, the effects of infestations on tree mortality varies according to the initial condition of the stand and the number of infestations. Davidson et al. (1999) found that stands with good crown condition, had mortality rates of only 7% and 22% after one and two infestations, respectively; however, in stands with poor crown quality, the corresponding mortality rates were 36% (one infestation) and 55% (two infestations). Heavy defoliation usually increases mortality rates even among trees that are generally not preferred by the gypsy moth as food sources. For instance, a single heavy defoliation in eastern New England resulted in 69% mortality of trees that are eaten but not preferred by the gypsy moth compared with about 37% mortality in oaks, a source of food that is preferred by the gypsy moth (Campbell and Sloan 1977b).

Gypsy moth infestations generally result in mortality losses of less than 15% of total basal area. For example, in an artificially induced gypsy moth outbreak in poplars (*Populus euramericana*) that resulted in nearly complete (70-100%) defoliation of some stands as well as a 25% decrease in stem production, tree mortality ranged from 6 to 10% (Agrawal et al. 2002). Losses of 15-35% are not uncommon, and losses occasionally exceed 50% (Gottschalk et al. 1987). Volume growth is reduced among surviving trees for approximately 3 years after a defoliation episode (Picolo and Terradas 1989; Twery 1991; Muzika and Liebhold 1999). The study by Twery (1987) indicates that, on average, stem volume growth in oaks decreased 20% in any year in which a tree was defoliated, compared with the previous year in which there was no defoliation. This effect is due in part to the reduced leaf area in the recovering trees (Wargo 1981a). In any given stand, heavy defoliation year after year tends to be a rare event. When such an event does occur, however, consequent tree mortality rates may become very high. In the area described by Campbell and Sloan (1977b), for example, only 7% of the mixed oaks rated "good" died following a single heavy defoliation. After two successive heavy defoliations, however, mortality rates in this category increased to 27%.

Between 1911 and 1921, defoliation caused by the gypsy moth was heavy along the eastern seaboard of New England. During this decade the oak component suffered about 60% mortality. About 30% of red maples and 33% of white pines also died (Campbell and Sloan 1977b). During the next decade, both defoliation and the responses to it were significantly less (Baker 1941; Campbell and Sloan 1977b). Tree mortality in response to gypsy moth outbreaks appears to follow a general pattern in which the most severe tree mortality occurs during and after an initial outbreak (Gansner and Herrick 1984; Herrick and Gansner 1986, 1988; Twery and Gottschalk 1989; Twery 1991). Campbell and Sloan (1977b) observed that certain trees within any given species consistently suffered heavier defoliation than others and were more likely to die, suggesting that differential intraspecific mortality could account for the subsequent decrement in stand vulnerability. Similarly, Byington et al. (1994) noted marked difference in tolerance to gypsy moth damage among nine families of red oak.

   4.1.2.5.2. Differential Feeding Preferences for Trees – Gypsy moth larvae appear to have definite food preferences; oak, birch, poplar, willow and apple trees seem to be their favorite food sources. In the northeast, preferences vary among species of oak with the greatest preference shown for black and burr oak (Foss and Rieske 2003). Other species, like beech, maple, and white pine are less favored by the gypsy moth, and hemlock and pitch pine seldom serve as food sources. Mortality in white pine, however, can be substantial in stands where pine occurs in the understory. Much less damage occurs in oak/pine stands where pine shares the canopy with oak (Brown et al. 1988). Other species of trees such as black locust and ash are generally not substantially damaged by the gypsy moth (Campbell 1979). The avoidance of green ash (*Fraxinus pennsylvanica*) by gypsy moth larvae seems to be related to the presence of chemicals—not clearly identified—in the leaves of the trees (Markovic et al. 1996). On the other hand, gypsy moth larvae seem able to adapt to even unsuitable hosts such as the alkaloid rich foliage of locust trees (Lazarevic et al. 2002).

In addition to general host preferences, there appear to be regional differentiations among preferred moth host plants. Oak is the preferred species in the east and quaking aspen is the preferred species in the Midwest (Redman and Scriber 2000). Hornbeam is strongly preferred by gypsy moth larvae in Quebec, but in New England it is only an intermediate host, while gray birch and quaking aspen are both preferred by gypsy moth larvae in New England but are classed as only as intermediate hosts in Quebec (Mauffette et al. 1983).

Compared with the European gypsy moth, the Asian gypsy moth feeds more voraciously and grows faster on white oak, larch, and paper birch. In the former Soviet Union, the Asian gypsy moth feeds on more than an estimated 600 tree or plant species. Moreover, the Asian gypsy moth may not only thrive on the same tree species eaten by the European gypsy moth, but may do better on many species that the European gypsy moth does not favor, such as the Douglas-fir (USDA/FS 1992). Waller (1994) reports that the Asian gypsy moth grows better than European gypsy moth on 50 plant species in the United States, with the greatest differences in growth rates associated with coniferous species (Wallner 1994). At least with the Asian gypsy moth, drought may be a predisposing factor to severe damage from infestations (Koltunov and Andreeva 1999).

The Asian strain of gypsy moth is a more serious problem in western forests, compared with the European strain (Montgomery 1993).

4.1.2.5.3. **Effects on Stand Structure and Productivity** – Subdominant trees (trees growing largely in shaded areas) suffer much higher rates defoliation induced mortality compared with the taller dominants after heavy defoliation (Brown et al 1988; Campbell 1979; McGraw et al. 1990; Quimby 1993). Usually, heavy and repeated defoliation results in a more one-storied stand. Although oak growing-stock volume in trees less than 10 inch diameter at breast height actually decreased between 1965 and 1989, the losses were offset by gains in larger trees (Gansner et al. 1993).

Hix et al. (1991) reported increases in red maple, which corresponded with decreases in oak trees, after defoliation in Appalachian forests. Moreover, total density increased from approximately 42,000 to 62,000 stems per acre, which the investigators attributed to the increase in light, nutrients, and moisture reaching the forest floor after defoliation. Regeneration in the Allegheny Mountain region is dominated by red maple, while red maple and birch dominate the Ridge and Valley regions. Oak reproduction is sparse and seedlings are small, compared with red maple and non-commercial seedlings, in the Allegheny Mountains. According to Allen and Bowersox (1989), only 4-16% of the stems were northern red oak or white oak. In many heavily defoliated stands, oak reproduction, which greatly depends on the survival of acorns and small oak seedlings (0-1 ft tall) is a major concern, especially given the limited information regarding the ability of oak to compete successfully with birch and maple trees (Twery 1991; Galford et al. 1993; Hix et al. 1991).

Moderately heavy defoliation usually accelerates forest succession toward more shade-tolerant (and less defoliation-prone) species (Campbell and Sloan 1977b; Houston 1981b). In contrast to widespread scarcity of oak regeneration in other infested areas, oaks often continue to dominate stands in frequently defoliated areas with excessively drained, sandy soils (e.g., Cape Cod, MA, and the New Jersey coastal plain) or rocky, shallow, ridge top soils (e.g., those common to Medford, MA). Other sources indicate that a small number of oaks in young stands in central New England may become dominant when the stands reach 50 years of age (Oliver 1978; Twery 1991).

Changes in forest composition may account for the frequently-cited reductions in gypsy moth-induced effects in areas such as New England. Gottschalk (1994) reports that moderate to heavy overstory mortality in recent years followed heavy defoliation on about 5-20% of defoliated Appalachian stands. Nevertheless, tree mortality rates in these stands show no indication of downturn after a second wave of equally heavy defoliation.

Even in healthy stands with little defoliation, heavy crops of hard mast (primarily acorns) are only produced about every 3 or more years and during intervening years, mast crops may be poor or nearly absent (USDA/FS 1994). Defoliation can virtually eliminate oak mast production, especially in the short-run and result in several consecutive years of complete mast failure

(Gottschalk 1990b; Liscinsky 1984). During years of moderate and heavy defoliation, short-term and residual adverse effects on mast production can be attributed to three sources: direct consumption of flowers; abortion of immature acorns due to a low carbohydrate supply; and lack of flower bud initiation. Available data suggest that abortion of immature acorns is the most significant of the adverse effects, which can result in up to 5 years of complete acorn failure (Gottschalk 1990a).

As previously noted, trees that do not die during a defoliation episode may take as long as 10 years to recover their full vigor. On the other hand, once trees recover their vigor, significant overstory mortality (>60% of the basal area) in intermediate and suppressed trees (i.e., not heavy mast producers) is required to cause significant reductions in acorn production. Acorn production by surviving trees may even be stimulated by this thinning (Gottschalk, 1990a). In the long term, loss of acorn and other nuts is partially compensated by an increase in the number of flowers (Gottschalk 1990b).

      4.1.2.5.4. Effects on Shrubs and Herbs – When heavy defoliation is followed by massive overstory mortality, existing shrub and herb cover increases dramatically due to increases in available light, moisture, and nutrients. Under certain conditions, heavy defoliation and subsequent overstory mortality can result in dominance by shrubbery and herbaceous vegetation for several years. Gansner (1985) describes an understory 10 years following defoliation as being dominated by blueberry, witch-hazel, raspberries, and several species of ferns, along with some tree seedlings that were heavily browsed by deer. Hix et al. (1991) also noted that blueberries and raspberries were often the shrub species that increased the most following defoliation. Among herbaceous plants, Brackley (1985) noted that gypsy moth-induced defoliation appeared to stimulate flowering in the endangered orchid, *Isotria meleoloides*, in New Hampshire.

      4.1.2.5.5. Effects on Fire Hazard – Researchers generally agree that heavy defoliation caused by the gypsy moth increases fire danger, although differences in fuels have not been measured and the increased fire hazard has not been calculated (Gottschalk 1990b). Wildfires are more difficult to control in areas of extensive tree mortality (Tigner 1992). Furthermore, the numerous standing dead snags may act as lightning rods and further increase risk of fire starts by lightning. Fire caused by a lightning strike on one or more of these snags could smolder for several days prior to detection (USDA/FS 1994). On the other hand, fires are infrequent during the growing season in eastern hardwood forests. Consequently, significant increases in fire hazard would occur in hardwood forests during the growing season only as the result of long-term increases in woody fuels due to tree mortality (Gottschalk 1990b).

4.1.2.6. Soil and Terrestrial Microorganisms – There is little information from which to directly assess the effect of gypsy moth infestations on terrestrial microorganisms. Indirect evidence suggests that adverse effects are unlikely. Soil microbial activity is largely influenced by moisture and temperature. Vaughan and Kasbohm (1993) report that defoliation increases maximum daily temperatures. Since microbial activity increases with temperature, defoliation is

likely to increase microbial activity, thereby accelerating the process of decomposition. Decomposing bacteria and fungi have high nutrient requirements. Increased nutrient content in litter fall might enhance decomposition, which might be the case during gypsy moth defoliation in the spring when leaf matter is consumed before nutrient reabsorption takes place (Grace 1986). The effects of these increased nutrient levels and mineralization might be to enhance forest regeneration.

4.1.3. Aquatic Organisms.

4.1.3.1. Fish – Little information is available regarding the effects of gypsy moth infestations on fish populations. Defoliation from the gypsy moth can result in an increase in the pH and temperature of ambient water (Downey et al. 1994; Webb et al. 1995). Downey et al. (1994) have suggested that trout, which as are very sensitive to changes in pH and temperature, could be adversely affected by such changes. As discussed by Webb et al. (1995), however, no direct data are available on the biological effects of such changes due to defoliation by the gypsy moth and it is unclear if significant biological effects on fish are likely.

4.1.3.2. Aquatic Invertebrates – Hutchens and Benfield (2000) detected an apparent increase in the rate of leaf breakdown in streams due to gypsy moth defoliation, which might result in food deficits during spring for shredders—i.e. caddisflies, stoneflies, and some dipterans. The number of shredders collected by Hutchens and Benfield (2000), however, was greater in disturbed streams (i.e., streams in areas of gypsy moth defoliation) than in control streams.

4.1.3.3. Aquatic Plants – Information directly related to the effects of gypsy moth infestations on algae in streams is available in the study by Sheath et al. (1986), which was conducted in a spring-fed, headwater stream in central Rhode Island from 1979 to 1982. In the first two summers, a dense riparian canopy reduced surface light penetration to a range of 5-18% of incident radiation, and the stream macroalgal community consisted of only one to four species covering from less than 1 to 35% of the stream bottom. In 1981, the surrounding leaf canopy was removed by a massive gypsy moth larval outbreak, which increased light penetration at the stream surface to 73% and increased the water temperature by 3.7°C. By early August, macroalgal cover increased to a peak of 80% of the stream bottom. A less severe gypsy moth defoliation in 1982 that did not have a significant impact on light, temperature or macroalgal cover from 1979 and 1980. In contrast, investigators on a stream in Shenandoah National Park observed no significant changes in periphyton abundance due to defoliation. These investigators speculated that many southern Appalachian streams are so low in nutrients that increased sunlight penetration alone is not enough to increase algal growth (USDA/FS 1994).

4.1.3.4. Aquatic Microorganisms – Particularly in small, first-order streams, defoliation by the gypsy moth provides increased sunlight at the water surface that may enhance microbial activity secondary to an increase in temperature (Sheath et al. 1986). As discussed in Section 4.1.3.2, defoliation by the gypsy moth appears to increase the rate of leaf breakdown in streams, which is due, in part, to greater microbial conditioning in leaf packs (Hutchens and Benfield 2000). Other major increases occur in fecal coliform and fecal streptococci densities in streams during periods

of maximum defoliation (Corbett and Lynch 1987). These increases might be associated with increases in nutrients from water contamination by frass and leaf fragments.

4.2. EXPOSURE ASSESSMENT
4.2.1. Overview
As in the human health risk assessment, the exposure metameter is dictated by the data used to formulate the dose-response assessment. Also as in the human health risk assessment, egg mass density is the exposure metameter for terrestrial invertebrates and plants because it is the measure on which the dose-response assessment is based. Egg mass densities spanning a range from 5 egg masses/acre to 5,000 egg masses per acre are used to estimate responses in terrestrial plants and invertebrates.

Most wildlife species are not affected directly by exposure to the gypsy moth but are more likely to experience indirect effects like changes in habitat or other environmental conditions secondary to defoliation. Consequently, the exposure assessment for most wildlife species is almost identical to the dose-response assessment for terrestrial plants which is expressed as defoliation caused by gypsy moth larvae. For this exposure assessment, categories of defoliation are defined normal background defoliation (<30% defoliation), moderate defoliation (30-60% defoliation), and high or severe defoliation (>60% defoliation).

4.2.2. Direct Exposure
As discussed in Section 4.1, the gypsy moth has a direct impact on terrestrial vegetation and certain terrestrial invertebrates, and the direct effects, especially the effects on vegetation, are likely to cause indirect effects in other organisms (Section 4.2.3). Like the exposure assessment for human health (Section 3.2), the exposure assessment for terrestrial vegetation and terrestrial invertebrates can be based on any of several exposure measures, including numbers of egg masses per acre, numbers of larvae per acre or tree, or larval mass per acre or tree. The various exposure measures are not necessarily correlated and may relate to more than vegetation damage.

Sample et al. (1996) assayed egg mass density, number of larvae per 50 g dry weight of vegetation, and defoliation in sets of stands: control stands with no significant level of gypsy moth infestation and no pesticide, stands with significant levels of gypsy moth infestation and no pesticide, stands with both gypsy moth infestation and treatment with *B.t.k.*, and stands treated with *B.t.k.* in the absence of significant gypsy moth infestation. Each set of stands consisted of six replicates of 50-acre plots in which measurements were made over a 3-year period. As illustrated in Figure 4-1, the relationship between egg mass density and the number of larvae per unit vegetation is extremely weak and scattered. More recently, Naidoo and Lechowicz (2001) published the results of a 13-year study in which they assayed the number of gypsy moth larvae per tree in different tree species in a forest in Quebec. Figure 2 in the study shows substantial variation in the numbers of larvae in different tree species in the forest. Red oak trees were the most heavily infested (up to 250 larvae per tree), and white ash trees were the least infested (maximum of four larvae per tree). This study clearly illustrates that gypsy moth larvae feed preferentially on different types of vegetation, resulting in substantial variation in infestation among tree species. Although larval density may be the most intuitively reasonable measure of exposure (i.e., to the gypsy moth larvae), the poor correlation of egg mass density to larval density noted in the study by Sample et al. (1996) may be due partly to larval feeding preferences

or dispersal. A complicating factor, discussed further in Section 4.3, is that larval counts themselves do not necessarily predict defoliation uniformly across different species of trees.

As in the human health risk assessment, the exposure metameter is dictated to some extent by the data used to formulate the dose-response assessment. In the human health risk assessment, the dose-response assessment is based on egg mass density (see Section 3.3); therefore, egg mass density is, by definition, the exposure metameter. As discussed in Section 4.3 and summarized in Table 4-1, egg mass densities spanning a range from 5 egg masses/acre to 5,000 egg masses per acre are used to estimate responses in terrestrial plants and invertebrates.

4.2.3. Indirect Exposure
As summarized in Section 4.1, most wildlife species are not affected directly by exposure to the gypsy and are more likely to experience the effects of indirect exposure like changes in habitat or other environmental conditions secondary to defoliation. Consequently, the exposure assessment for most wildlife species is almost identical to the dose-response assessment for terrestrial plants (Section 4.3) – i.e., the assessment is expressed as defoliation caused by gypsy moth larvae. Defoliation can be categorized various ways, all of which are largely judgmental. For example, Agrawal et al. (2000) define light defoliation as 20-40%, severe defoliation as 40-90%, and nearly complete defoliation as 75-100%. The categories used in the previous EIS as well as in the study by Gottschalk et al. (1998) are used in the current risk assessment: normal background defoliation (<30% defoliation), moderate defoliation (30-60% defoliation), and high or severe defoliation (>60% defoliation).

The use of these somewhat arbitrary categories has only a minimal impact on the current risk assessment, which is largely qualitative since the available data do not permit quantitative measures of response as a function of defoliation for most wildlife species. This issue is discussed further in the risk characterization (Section 4.4).

4.3. DOSE-RESPONSE ASSESSMENT
4.3.1. Overview
The quantitative dose-response assessment for the gypsy moth is illustrated in Figure 4-2. As in the human health risk assessment, the dose metameter is egg masses/acre. Quantitative dose-response assessments can be made for both terrestrial plants and sensitive species of lepidoptera. The dose-response assessments for terrestrial plants are based on a relatively simple quantitative model for the relationship of egg mass density to defoliation. Three broad categories (sensitive, intermediate, and tolerant) are used to characterize the susceptibility of forest stands to gypsy moth induced defoliation. Estimated LOAEL values based on 30% defoliation, which is considered the lower range of moderate defoliation, are approximately 125, 1000, and 7000 egg masses/acre for sensitive, intermediate, and tolerant forest stands, respectively. The corresponding NOAEL values, defined as 10% defoliation, are estimated as 12, 20, and 125 egg masses/acre for sensitive, intermediate, and tolerant forest stands.

The gypsy moth may affect some sensitive terrestrial invertebrates, including some species of lepidoptera. These effects, however, are less well documented and less well characterized compared with the effects on terrestrial plants. Nonetheless, available studies indicate that the NOAEL for adverse effects in certain other species of lepidoptera are lower than the NOAEL for sensitive forest stands—— i.e., about 6-72 egg masses/acre for some lepidoptera.

No quantitative dose-response assessment is presented for other groups of organisms—e.g., mammals, birds, and soil or aquatic organisms. The impact of gypsy moth exposure on these species is most likely to result in indirect effects (i.e., secondary to defoliation). Qualitative expressions of risk for these species are presented in the Risk Characterization (Section 4.4.4).

4.3.2. Terrestrial Plants
In terms of assessing direct effects of the gypsy moth on terrestrial vegetation, the most common endpoint used to express damage is defoliation. As discussed in Section 4.1.2.5, numerous factors, many of which are interrelated, influence the level of damage that gypsy moth larvae may cause to a forest stand or region. Several models are useful for quantifying the likely levels of defoliation (e.g., Davidson et al. 2001; Gansner et al. 1985; Gottschalk et al. 1998; Gribko et al. 1995; Liebhold et al. 1993; Montgomery 1990;Weseloh 1996a; Williams et al. 1991). Furthermore, the USDA Forest Service developed an expert system, GypsES, to facilitate the use of modeling in the management of gypsy moth programs (Gottschalk et al. 1996; Williams et al. 1997; http://www.fs.fed.us/na/morgantown/fhp/gypses /gypmain.htm).

The common exposure factor for all of these models is egg mass density. As discussed in Section 3.1, egg mass density is usually measured during the fall to predict damage in the following season. This indicator allows individuals involved in gypsy moth control programs enough time to plan an intervention strategy before damage occurs. The models, some of which are extremely complex, incorporate several factors, in addition to egg mass density, that affect defoliation—e.g., types of vegetation, terrain characteristics, and various geographical characteristics. For example, Weseloh (1996a) developed a 23-parameter model that

incorporates egg mass density, latitude, longitude, elevation, drainage, factors for the history of defoliation over a previous 4-year period, and 12 parameters for interaction terms. The model was developed using data from defoliation patterns in Connecticut from 1987 to 1994. When used retrospectively on defoliation rates from 1974 to 1986, the model was reasonably accurate in predicting years of heavy defoliation (correlations of up to 0.8) but less accurate in predicting years with relatively little defoliation (correlations on the order of 0.2).

For the current risk assessment, the relatively simple four-parameter model developed by Davidson et al. (2001) seems most useful for describing key factors that will have an impact on defoliation:

$$DEF_S = (BA_S/BA_T)^{b1} \times BA_p^{b2} \times \ln(EM)^{b3} \times Y \times e^{\gamma Y}$$

where:

Y	=	number of years since start of outbreak
DEF_S	=	percent stand defoliation at time Y of outbreak
BA_S	=	basal area of sensitive species in stand (m^2/ha)
BA_P	=	basal area of pine species in stand (m^2/ha)
BA_T	=	total basal area in stand (m^2/ha)
EM	=	egg masses per hectare

and γ, b1, b2, and b3 are model parameters. Based on defoliation and egg count data collected over 4 years (1992 through 1995) from seven pine-oak stands and nine pine-sweetgum stands in Maryland, Davidson et al. (2001) estimated the following values for the model parameters: 0.2226 for b1, -0.2684 for b2, 2.0792 for b3, and -0.5781 for γ. Notably, the parameters associated with the ratio of sensitive tree species (BA_S/BA_T) and egg mass density (EM) are positive indicating that damage increases as these model components increase. The parameters associated with the basal area of pine species (BA_p) as well as the exponential function for years since the start of the outbreak ($e^{\gamma Y}$) are negative. In other words, an increase in the density of pine species (trees generally not favored by the gypsy moth) will tend to reduce defoliation and the outbreak will subside over time. All of these factors in the model are qualitatively consistent with the behavior of gypsy moth infestations (see Section 4.1.2.5).

The model developed by Davidson et al. (2001), though relatively simple, is still multidimensional, which means the estimates of defoliation depend highly on site specific factors. Any number of defoliation estimates could be made based on varying any of the input variables in the model by Davidson et al. (2001). For this risk assessment, three general types of forest stands are considered: sensitive, intermediate, and tolerant. Each stand is assumed to have a total basal area of 15 m^2/ha. This is somewhat arbitrary but since the total basal area is only used as a normalizing factor on the basal area of sensitive species, this assumption has no impact on the model. The basal surface area for sensitive species is taken as 13 m^2/ha for sensitive stands, 6 m^2/ha for intermediate stands, and 2 m^2/ha for tolerant stands. The basal area for pine is taken as 0.25 m^2/ha for sensitive stands, 1.5 m^2/ha for intermediate stands, and 3 m^2/ha for tolerant stands. The percent defoliation in all stands is calculated for 1 year from the initial time

of the outbreak. Egg mass density is modeled over a range from 1 egg mass/ha (approximately 0.4 egg masses per acre) to 25,000 egg masses/ha (approximately 10,000 egg masses per acre). Again, despite the arbitrary nature of these ranges and assumptions, they reflect the variability of and responses among the stands considered in the Davidson et al. (2001) publication.

The variability of responses in the three different stand types is illustrated in Figure 4-2. The curved lines indicate the percent defoliation expected in sensitive, intermediate, and tolerant stands over the range of egg mass densities considered. The two thick horizontal lines represent breakpoints for the classifications of defoliation discussed in Section 4.2.3—i.e., normal background defoliation (<30% defoliation), moderate defoliation (30-60% defoliation), and high or severe defoliation (>60% defoliation). Sensitive stands are likely to show evidence of low level intermediate defoliation—i.e., 30% defoliation—at an egg mass density of about 125 egg masses/acre. Comparable values for intermediate and tolerant stands are about 1000 egg masses/acre and 7000 egg masses/acre, respectively. For sensitive stands, the egg mass density associated with 50% defoliation is about 600 egg masses/acre and this estimate is consistent with field observations for sensitive stands (Montgomery 1990).

Risks to wildlife species from most agents used to control the gypsy moth are based on NOAEL values (no observed adverse effect levels) and LOAEL values (lowest observed adverse effect levels). As discussed in Section 4.2.3, 30% defoliation is used in this risk assessment as a border value between background and moderate defoliation. Thus, the egg mass densities of 125, 1000, and 7000 egg masses/acre for sensitive, intermediate, and tolerant stands, respectively, are essentially LOAEL values—the lowest egg mass density that might be associated with a level of defoliation classified as moderate or a minimal adverse effect.

The model by Davidson et al. (2001) as well as other models for gypsy moth defoliation are non-threshold. In other words, any level of exposure is assumed to carry some risk. Thus, the selection of a functional NOAEL is somewhat arbitrary. Following the general approach used to estimate a benchmark dose (U.S. EPA 2001), the defoliation rate of 10% is used as a functional NOAEL. Based on the dose-response curves illustrated in Figure 4-2, these NOAEL values are egg mass densities of about 12 egg masses/acre for sensitive stands, 20 egg masses/acre for intermediate stands, and approximately 125 egg masses/acre for tolerant stands.

These NOAEL and LOAEL estimates are at best crude approximations of egg mass densities associated with levels of defoliation that might be considered essentially benign (NOAELs) or capable of causing detectable damage (LOAELs) in various forest stands. The primary use of these values is to provide a basis for comparing risks associated with exposure to the gypsy moth to risks associated with exposure to agents used to control the gypsy moth.

4.3.3. Terrestrial Invertebrates
The impact of gypsy moth exposure on terrestrial invertebrates cannot be quantified. As discussed in Section 4.1.2.4, a few relatively recent studies demonstrate that exposure to gypsy moth larvae during an outbreak may adversely affect some other lepidopterans (Sample et al.

1996; Work and McCullough 2000). Furthermore, the study by Redman and Scriber (2000) suggests that at least some of these effects could be related directly to gypsy moth exposure rather than to effects secondary to gypsy moth-induced defoliation.

Although the data on invertebrates are limited in terms of defining a quantitative exposure-response relationship, the study by Sample et al. (1996) defines an apparent NOAEL of 6-72 egg masses/acre for effects on tiger moths. The corresponding LOAEL is 95-468 egg masses/acre and is associated with decreases in the abundance of tiger moth larvae and adults. These values are illustrated in Figure 4-2 as the mean of each range rounded to one significant place—i.e, a value of 40 egg masses/acre for the NOAEL and 300 egg masses/acre for the LOAEL.

4.3.4. Other Species
As discussed in Section 4.2.3, other species may be affected by gypsy moth infestations secondary to defoliation. These observations, as summarized in Section 4.1 (Hazard Identification), are essentially qualitative—i.e., the effects were observed in the field or are based on plausible assumptions in cases of severe gypsy moth outbreaks and extensive defoliation. Thus, no quantitative dose-response assessment is proposed for species that are indirectly affected, and the plausible responses are discussed qualitatively in the risk characterization (Section 4.4.4).

4.4. RISK CHARACTERIZATION
4.4.1. Overview
The best documented and most obvious effect of the gypsy moth will be defoliation of terrestrial vegetation, particularly in forest stands in which sensitive species of trees predominate. In some respects, the risk characterization for terrestrial vegetation is essentially a restatement of the hazard identification. In other words, the effects of gypsy moth larvae on forests is extremely well documented and relatively well understood. In sensitive forest stands—i.e., stands in which oak, birch, and other favored species predominate —gypsy moth larvae can cause substantial defoliation. In forest stands in which tree species that are not favored by gypsy moth larvae predominate —e.g., hemlock, various types of pine, black locust and white ash—even relatively high exposures, as measured by egg mass density, may not result in substantial defoliation. The risk assessment for direct effects on forests should be at least qualitatively influenced by the current range of the gypsy moth, which has not yet extended to some forests in the southeast that may be among the most sensitive to the gypsy moth. Thus, unless measures to contain the gypsy moth are successful, these southeastern forests may suffer serious damage in future infestations.

Some other lepidopteran species also may be directly affected by exposure to the gypsy moth. Most studies, however, suggest that substantial adverse effects in terrestrial insects are unlikely and effects in some insect species, including some other lepidoptera, may be beneficial.

Because the gypsy moth may substantially damage some forests in severe infestations or outbreaks, secondary effects in other species of wildlife are plausible. Reductions in populations of squirrels, mice, and other mammals which may be sensitive to changes in the availability of acorns are likely. Substantial adverse effects on other groups of animals – i.e., birds, reptiles, and aquatic species – cannot be ruled out but have not been convincingly demonstrated.

4.4.2. Direct Effects
4.4.2.1. Terrestrial Plants – A quantitative summary of the risk characterization for forest stands is presented in Table 4-1. Risks are expressed as hazard quotients and estimates of percent defoliation for three classes of forest stands: sensitive, intermediate, and tolerant. As discussed in Section 4.3.2, these classifications are intended to reflect, albeit crudely, differences in susceptibility of various forest stands to the effects of gypsy moth exposure, which is predicated on the feeding preferences of gypsy moth larvae. The specific types of trees favored and not favored by the gypsy moth are discussed in Section 4.1.2.5.2. The NOAEL values and quantitative estimates of defoliation are based on the dose-response model proposed by Davidson et al. (2001). Although the dose-response model is relatively simple, it does appear to reflect the variables that have the most significant impact on the sensitivity of various forest stands to defoliation by gypsy moth larvae. The four categories of infestations used in the dose-response assessment are based on egg mass densities of 5, 50, 500, and 5000 egg masses/acre. These categories generally encompass the range of egg mass densities reported in the literature for infestations ranging in degree from negligible to severe and are similar to the categories used in the human health risk assessment (Table 3-3). The hazard quotients presented in Table 4-1 will be used primarily in the comparative risk assessment, which is a separate document that provides

a quantitative comparison of the risks for each of the various agents used to control the gypsy moth as well as the risks posed by the gypsy moth itself.

In some respects, the risk characterization for terrestrial vegetation is essentially a restatement of the hazard identification. In other words, as discussed in Section 4.1.2.5, the effects of gypsy moth larvae on forests is extremely well documented and relatively well understood. In sensitive forest stands—i.e., stands in which oak, birch, and other favored species predominate —gypsy moth larvae can cause substantial defoliation. In forest stands in which tree species that are not favored by gypsy moth larvae predominate —e.g., hemlock, various types of pine, black locust and white ash—even relatively high exposures, as measured by egg mass density, may not result in substantial defoliation.

The quantitative risk characterization focuses on defoliation; however there are likely to be other effects of gypsy moth exposure. In some cases, extensive defoliation will result in tree mortality. In general, however, tree mortality is likely to be relatively low (on the order of 10-20%), although higher rates of mortality (up to about 50%) are possible when sensitive species are subject to multiple years of defoliation (see Section 4.1.2.5.1). Defoliation and tree mortality can lead to secondary effects of exposure on animals (see Section 4.4.3). Likewise, non-target vegetation may be subject to secondary effects of exposure, such as increases in understory growth (see Section 4.1.2.5.4). The extent to which the secondary effects are considered beneficial or detrimental depends to some extent on forest management objectives which are beyond the scope of this risk assessment.

The risk assessment for direct effects on forests should be at least qualitatively influenced by the range of the gypsy moth. That range has not yet extended to some forests that may be among the most sensitive to gypsy moth exposure. Many forests in the southeast and midwest are populated with a high proportion of tree species that are favored by the gypsy moth. Unless measures to contain the gypsy moth are successful, these regions may suffer serious damage from future infestations by the gypsy moth (Liebhold and McManus 1999).

4.4.2.2. Terrestrial Invertebrates – There is plausible concern regarding direct effects of the gypsy moth on some lepidopteran species. Nonetheless, few studies support this concern relative to the large number of studies regarding effects on terrestrial plants. As summarized in 4.3.3, the study by Sample et al. (1996) suggests a NOAEL of about 40 egg masses/acre for Arctiidae larvae and adults in terms of abundance and species richness. The direct effect of the gypsy moth, however, involved only lepidoptera from a single family, Arctiidae, which includes the tiger moths. No effects were seen in other lepidopteran or non-lepidopteran species; nonetheless, the effects observed on Arctiidae adults and larvae were statistically significant. Based on the approximate NOAEL of 40 egg masses/acre, this family of lepidoptera would still be less sensitive to gypsy moth larvae than most forest stands.

It is difficult to assess the extent to which other lepidopteran or non-lepidopteran groups might be affected by exposure to gypsy moth larvae. Redman and Scriber (2000) report several

mechanisms associated with the adverse effect of gypsy moth larvae on the northern tiger swallowtail butterfly, *Papilio canadensis*. There are, however, no other field studies that suggest the plausibility of substantial adverse effects in most insect species during gypsy moth infestations. In addition, gypsy moth induced defoliation may be beneficial to some butterfly species (Schweitzer 1988) or will have no effect on most other insect species (Sample et al. 1996).

4.4.3. Indirect Effects
4.4.3.1. Terrestrial Mammals – There is only limited information regarding the potential effects of gypsy moth infestations on mammalian wildlife. Adverse effects on reproduction or nutritional status were not observed in black bears after exposure to the gypsy moth during an outbreak that caused substantial mortality in oak trees and decreased acorn production (Vaughan and Kasbohm 1993). As noted in Section 4.1.2.1, bears adjusted for the decrease in acorn production by switching to alternate foods. It is not clear, however, that all mammals would adapt to a severe decrease in hard mast production. Consequently, there is plausible concern about the potential for adverse effects (reductions in populations) in squirrels, mice, and other mammals which may be sensitive to changes in the availability of acorns.

4.4.3.2. Birds – The effects of gypsy moth exposure on birds appear to be highly variable, with reports of both detrimental effects as well as beneficial effects (see Section 4.1.2.2). While the available data are not amenable to quantitative analyses, it would appear that these mixed effects are likely to be most pronounced during severe infestations.

4.4.3.3. Amphibians and Reptiles – There are no studies that clearly indicate adverse effects in either amphibians or reptiles after exposure to the gypsy moth. As discussed in Section 4.1.2.3, substantial defoliation of mast producing trees might adversely affect snakes that consume small mammals (e.g., squirrels and mice), the populations of which might decrease due to mast failure (see Section 4.4.3.1).

4.4.3.4. Terrestrial Microorganisms – There is no information regarding the effects of the gypsy moth or gypsy moth defoliation on terrestrial microorganisms. Intuitively, it seems reasonable that soil microbial activity would increase in response to defoliation as a result of the subsequent increase in ground temperature and nutrient load that would result from increases in litter and frass production (see Section 4.1.2.6). These effects, although not reported in the literature for soil microbial activity, are reported for aquatic microorganisms (see Section 4.1.3.4).

4.4.3.5. Aquatic Organisms – There is very little information to indicate that gypsy moth infestations cause substantial adverse effects on aquatic organisms (see Section 4.1.3). Stream microorganisms are likely to be affected directly by gypsy moth infestations due to the potential increase of microbial activity in forest streams. The increased activity is mostly like due to the increased nutrient loading of streams which results from defoliation and larval frass. Although Hutchens and Benfield (2000) suggest that the increase in activity might cause a food deficit for aquatic insects that shred decomposing leaves, the investigators found that the population of such

organisms (i.e., caddisflies, stoneflies, and some dipterans) were higher in streams in areas of gypsy moth defoliation compared with streams in the control areas. Because of increased light and water temperature secondary to defoliation, algal and aquatic macrophyte growth is likely to be increased (see Section 4.1.3.3), which might increase productivity in some streams but adversely affect water quality and habitat characteristics in other streams.

5. REFERENCES

Aber R; DeMelfi T; Gill T; Healey B; McCarthy MA. 1982. Rash illness associated with gypsy moth caterpillars – Pennsylvania. Morbidity Mortality Weekly Report. 31: 169-170.

Abrahamson L; Klass. 1982. Gypsy moth. New York: New York Media Services, Cornell University. 13 pp.

Adams S; Senft D. 1994. The busiest of bees: pollen bees outwork honey bees as crop pollinators. Agric Research. pp. 9-12. Cited in USDA 1995b.

Agrawal AA; Kosola KR; Parry D. 2002. Gypsy moth defoliation and n fertilization affect hybrid poplar regeneration following coppicing. Canadian Jrn Forestry Res. 32: 1491-1495.

Allen AW. 1987. Habitat suitability index models: Gray squirrel revised. U.S. Fish Wildl Serv Biol Rep. 82(10.135) 16 p. Cited in USDA 1995b.

Allen D; Bowersox. T. 1989. Regeneration in oak stands following gypsy moth defoliation. In: G Rink and CA Budelsky, eds. Seventh Central Hardwoods Forest Conf. Proc. Carbondale, IL. USDA Forest Service Gen Tech Rep. NC-132:67-73. Cited in USDA 1995b.

Allen VT; Gredmiller O; Tyler WB. 1991. Gypsy moth caterpillar dermatitis revisited. J Am Acad Dermatol. 24(6/1): 979-981.

Alt GL. 1990. Reproductive biology of female black bears and early growth and development of cubs in northeastern Pennsylvania. Dissertation Abstracts Internet. Cited in USDA 1995b.

Anderson JF; Furniss WE. 1983. Epidemic of urticaria associated with first-instar larvae of the gypsy moth (Lepidoptera:. Lymantriidae). J Med Entomol. 20(2): 146-150.

Anonymous. 1984. Work-related allergies in insect-raising facilities. MMWR (Morbidity Mortality Weekly Report. 33(31): 448, 453-454. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/00000386.htm.

Auchmoody LR; Walters RS. 1992. Impact of deer browsing, overstory density, and composition on survival and development of planted northern red oak seedlings. HS Department of Agriculture Forest Serv Gen Tech Rep. Cited in USDA 1995b.

Baker WL. 1941. Effects of gypsy moth defoliation on certain forest trees. J Forestry. 39:1017-1022.

Baker RH. 1968. Habitats and distribution. In: King JA ed. Biology of *Peromyscus* (Rodentia). Am Soc Mammalogists Spec Publ. Cited in USDA 1995b.

Baker WL; Cline AC. 1936. A study of the gypsy moth in the town of Petershan, Mass in 1935. J Forestry. 34:140-147.

Baltensweiler W; Fischlin A. 1988. The larch budmoth in the Alps In: Berryman AA ed. Dynamics of forest insect populations: patterns, causes, implications. New York, Plenum Press. Cited in USDA 1995b.

Banaszak J. 1992. Strategy for conservation of wild bees in an agricultural landscape. Agric Ecosyst Environ. 40:179-192. Cited in USDA 1995b.

Barber HL. 1984. Eastern mixed forest. In: White-tailed deer ecology and management. Stackpole Harrisburg, PA. Cited in USDA 1995b.

Bardwell CJ; Averill AL. 1996. Effectiveness of larval defenses against spider predation in cranberry ecosystems. Environ Entomol. 25: 1083-1091.

Beaucher WM; Farnham ME. 1982. Gypsy-moth-caterpillar dermatitis. New Eng J Med. 306: 1301-1302.

Behre CE. 1939. The opportunity for forest practices in the control of gypsy moth in Massachusetts woodlands. J Forestry. 37(7):546-551.

Behre CE; Reineke LH. 1943. The opportunity for silvicultural control of the gypsy moth in Southwestern Maine. J Forestry. 41:811-815.

Bell JL; Whitmore RC. 1997a. Eastern towhee numbers increase following defoliation by gypsy moths. Auk. 114(4): 708-716.

Bell JL; Whitmore RC. 1997. Bird Populations and Habitat in *Bacillus thuringiensis* and Dimilin-treated and Untreated Areas of Hardwood Forest. Am Midl Nat. 137(2): 239-250.

Berryman AA. 1983. Forest insects: principles and practice of population management. New York, Plenum Press. 279 p. Cited in USDA 1995b.

Bess JA. 1961. Population ecology of the gypsy moth, *Porthetria dispar* (L.) (Lepidoptera: Lymantriidae). Conn Agric Exper Sta Bull. GH6: 56. Cited in USDA 1995b.

Bess HA; Spurr SH; Littlefield EW. 1947. Forest site conditions and the gypsy moth. Harvard For Bull 22. 56 p.

Bormann FH; Likens GE; Siccama TG; Pierce RS; Eaton JS. 1974. The export of nutrients and recovery of stable conditions following deforestation at Hubbard Brook. Ecol Monogr. Cited in USDA 1995b.

Brackley FE. 1985. The orchids of New Hampshire. Rhodora. 87:1-117. Cited in USDA 1995b.

Bromley SW. 1935. The original forest types of southern New England. Ecol Monogr. 5:61-89. Cited in USDA 1995b.

Brooks RT; Smith HR; Healy WM. 1998. Small-mammal abundance at three elevations on a mountain in central Vermont, USA: a sixteen-year record. Forest Ecology and Management. 110: 1-3, 181-193.

Brown RC; Sheals RA. 1944. The present outlook on the gypsy moth problem. J Forestry. 42:393-407.

Brown JH; Cruickshank VB; Gould WP; Husband TP. 1988. Impact of gypsy moth defoliation in stands containing white pine. Northern J Appl For. 5:108-111.

Burnett JW; Calton GJ; Morgan RJ. 1989. Caterpillar and moth dermatitis. Cutis. 37(5): 320.

Buss LJ.; McCullough DG.; Ramm CW. 1999. Comparison of three egg mass survey methods in relation to gypsy moth (Lepidoptera: Lymantriidae) defoliation in Michigan. Environ Entomol. 28(3): 485-495.

Byington TS; Gottschalk KW; McGraw JB. 1994. Within-population variation in response of red oak seedlings to herbivory by gypsy moth larvae. The American midland naturalist. 132 (2): 328-339.

Campbell RW. 1973. Numerical behavior of a gypsy moth population system. Forest Sci. 19(3): 162-167.

Campbell RW. 1976. Comparative analysis of numerically stable and violently fluctuating gypsy moth populations. Environ Entomol. 5:1218-1224.

Campbell RW. 1979. Gypsy moth: forest influence. USDA Forest Service Agriculture Information Bulletin No. 423. 45 p.

Campbell RW. 1981. Population dynamics. In: Doane CC and McManus ML eds. The gypsy moth: research toward integrated pest. management. USDA Forest Service Technical Bulletin 1584. Cited in USDA 1995b.

Campbell RW; Garlo AS. 1982. Gypsy moths in New Jersey pine-oak. J Forestry. 80:89-90.

Campbell RW; Sloan RJ. 1977a. Natural regulation of innocuous gypsy moth populations. Environ Entomol. 6:315-322.

Campbell RW; Sloan RJ. 1977b. Forest stand responses to defoliation by the gypsy moth. For Sci Monogr. 19:34. Cited in USDA 1995b.

Campbell RW; Sloan RJ. 1978a. Numerical bimodality among North American gypsy moth populations. Environ Entomol. 7:641-646.

Campbell RW; Sloan RJ. 1978b. Natural maintenance and decline of gypsy moth outbreaks. Environ Entomol. 7:389-395.

Campbell RW; Miller MG; Duda EJ; Biazak CE; Sloan RJ. 1976. Man's activities and subsequent gypsy moth egg-mass density along the forest edge. Environ Entomol. 5(2): 273-276.

CDC (Centers for Disease Control). 2004. Lyme Disease. http://www.cdc.gov/ncidod/dvbid/lyme/.

Christie I; Wilder JW; Colbert JJ. 1995. Modeling of one-dimensional spatial effects on the spread of gypsy moths. Ecological Modeling. 78 (3): 219-234.

Colbert JJ; Racin G. 1995. User's guide to the stand-damage model: A component of the gypsy moth life system model. Gen Tech Rep NE. (207): 38.

Colbert JJ; Xu R; Jiang NQ. 1995. A simplified gypsy moth model system: Model definition and description. Comput Electron Agric. 13(2): 115-131.

Cook SP; Smith HR; Hain FP; Hastings FL. 1995. Predation of gypsy moth (Lepidoptera: Lymantriidae) pupae by invertebrates at low small mammal population densities. Environ Entomol. 24: 1234-1238.

Cooper RJ; Smith HR. 1995. Predation on gypsy moth (Lepidoptera: Lymantriidae) egg masses by birds. Environ Entomol. 24: 571-575.

Corbett ES. 1991. Gypsy moth defoliation impacts on water quality and quantity. USDA Interagency Gypsy Moth Research Forum. University Park, PA. USDA Forest Service, Northeastern Forest Experiment Station. Cited in USDA 1995.

Corbett ES; Lynch JA. 1987. The gypsy moth – does it affect soil and water resources? In: Forbroke S and Hicks J, Eds. Coping with the gypsy moth in the new frontier. West. Virginia University, Morgantown. Cited in USDA 1995.

Cram WA. 1990. Gaining support for British Columbia's gypsy moth wars, 1978-1988. Pest Management Report No. 12. Vancouver, BC: British Columbia Ministry of Forests. Available from NTIS, Springfield, VA: MIC-91-03022. Cited in USDA 1995.

Crocoll S. 1991. The potential impact of gypsy moth defoliation on broad-winged hawk reproductive success. The Kingbird. 41:224-227.

Davidson CB; Gottschalk KW; Johnson JE. 1999. Tree mortality following defoliation by the European gypsy moth (*Lymantria dispar* L.) in the United States: A review. For Sci. 45(1): 74-84.

Davidson CB; Johnson JE; Gottschalk KW; Amateis RL. 2001. Prediction of stand susceptibility and gypsy moth defoliation in coastal plain mixed pine-hardwoods. Canadian J Forestry Res. 31: 1914-1921.

DeGraaf RM. 1987. Breeding birds and gypsy moth defoliation: Short-term responses of species and guilds. Wildl Soc Bull. 15:217-221.

DeGraaf RM; Holland DG. 1978. Response of breeding birds to gypsy moth defoliation of an upland oak forest. Trans Northeast Section Wildl Soc. 35:105-119. Cited in USDA 1995b.

Deml R.; Dettner K. 1995. "Balloon Hairs" of gipsy moth larvae: Morphology and comparative chemistry. Insect Biochem Mol Biol. 112(4): 673-681.

Doane CC. 1970. Primary pathogens and their role in the development of an epizootic in the gypsy moth. J Invertebr Pathol. 15:21-33.

Downey DM. 1991. Do gypsy moths impact stream water chemistry. In Appalachian Gypsy Moth Integrated Pest Management Demonstration Project News. Morgantown, WV. Cited in USDA 1995b.

Downey DM; Armstrong JD; Bennett KH; French CR; Kraul TW. 1994. Impact of watershed defoliation by gypsy moths: water chemistry changes in low ANC headwater streams. James Madison Univ. Cited in USDA 1995b.

Dunn JP; Kimmerer TW; Nordin GL. 1986a. Attraction of the twolined chestnut borer, *Agrilus bilineatus* and associated borers to volatiles of stressed white oak. Can Ent. 118:503-509.

Dunn JP; Kimmerer TW; Nordin GL. 1986b. The role of host tree condition in attack of white oaks by the twolined chestnut borer, *Agrilus bilineatus*. Oecologia 70:596-600.

Dunn JP; Kimmerer TW; Potter DA. 1987. Winter starch reserves of white oak as a predictor of attack by the twolined chestnut borer, *Agrilus bilineatus* (Weber) (Coleoptera: Buprestidae). Oecologia 74:352-355.

Elkinton JS; Liebhold AM. 1990. Population dynamics of gypsy moth in North America. Ann Rev Entomol. 35: 571-596.

Elkinton JS; Healy WM; Buonaccorsi JP; Boettner GH; Hazzard AM; Smith HR; Liebhold AM. 1996. Interactions among gypsy moths, white-footed mice, and acorns. Ecology. 77: 2332-2342.

Elkinton JS; Healy WM; Liebhold AM; Buonaccorsi JP; McShea WJ ;Healy WM. 2002. Gypsy moths and forest dynamics. Chapter 7 in: Oak forest ecosystems: Ecology and management for wildlife. pp. 100-112. WJ McShea and WM Healy, Eds. Johns Hopkins University Press, Baltimore, Maryland.

Erelli MC; Elkinton JS. 2000. Maternal effects on gypsy moth (Lepidoptera: Lymantriidae) population dynamics: A field experiment. Environ Entomol. 29: 476-488.

Eshleman KN; Morgan RP; Webb JR; Deviney FA; Galloway JN. 1998. Temporal patterns of nitrogen leakage from mid-Appalachian forested watersheds; role of insect defoliation. Water Resources. Washington: American Geophysical Union, Aug 1998. v. 34 (8) p. 2005-2016.

Etkind PH; O'Dell TM; Canada AT. 1982. The gypsy moth caterpillar: A significant new occupational and public health problem. J Occup Med. 24(9): 659-662.

Feldhamer GA. 2002. Acorns and white-tailed deer: Interrelationships in forest ecosystems. Chapter 14 in: Oak forest ecosystems: Ecology and management for wildlife. pp. 214-223. WJ McShea and WM Healy, Eds. Johns Hopkins University Press, Baltimore, Maryland.

Forbush EH; Fernald CH. 1896. The gypsy moth. Boston, MA: Wright and Potter Printing Co. Cited in USDA 1995a.

Foss LK; Rieske LK. 2003. Species-specific differences in oak foliage affect preference and performance of gypsy moth caterpillars. Entomol Exp Appl. 108: 87-93.

Fuester RW; Taylor PB. 1996. Differential mortality in male and female gypsy moth (Lepidoptera: Lymantriidae) pupae by invertebrate natural enemies and other factors. Environ Entomol. 25: 536-547.

Galford J; Auchmoody LR; Smith HC; Walters RS. 1993. Insects affecting establishment of northern red oak seedlings in central Pennsylvania. Eight Central Hardwood Forest Conf. pp. 271-280. Cited in USDA 1995b.

Gansner DA. 1985. Ten years after gypsy moth and still no regeneration. Pennsylvania Forests. 75:6, 12.

Gansner DA; Herrick OW. 1984. Guides for estimating forest stand losses to gypsy moth. North J Appl Forest. 1:21-23.

Gansner DA; Herrick OW; Ticehurst M. 1985. A method for predicting gypsy moth defoliation from egg mass counts. North J Appl Forest. 2:78-79.

Gansner DA; Arner SL; Widmann RH; Alerich CL. 1993a. After two decades of gypsy moth, is there any oak left?. North J Appl Forest. 19(4): 184-186.

Gerardi MH; Grimm JK. 1979. The History, Biology, Damage, and Control of the Gypsy Moth, *Porthetria dispar* (L.). Associated University Presses, Inc., London, England. 233 pp.

Gilmer PM. 1925. A comparative study of the poison apparatus of certain lepidopterous larvae. Ann. Entomol. Soc. Am. 28: 203-239.

Glaser RW; Chapman JW. 1913. The wilt disease of gypsy moth caterpillars. J Econ Entomol. 6:479-488.

Goldman L; Faye S; Levine A; et al. 1960. Investigative studies of skin irritations from caterpillars. J. Invest. Dermatol. 34: 67-79.

Gorman OT; Roth RR. 1989. Consequences of a temporally and spatially variable food supply for an unexploited gray squirrel (*Sciurus carolinensis*) population. Am Midland Nat. 121: 41-60.

Gottschalk KW. 1990a. Gypsy moth effects on mast production. In: McGee CE, ed. Proc Southern Appalachian Mast Management Workshop, August 14-16,. 1989, Knoxville, TN. Cited in USDA 1995b.

Gottschalk KW. 1990b. Economic evaluation of gypsy moth damage in the United States of America. In: Proc Division 4, IUFRO. Can IUFRO World Congress Organizing Comm. Cited in USDA 1995b.

Gottschalk KW; Gansner DA; Herrick OW; Mason GN. 1987. Coping with gypsy moth: guidelines for forest managers. In: Economic and social development: a role for forests and forestry professionals. Society of. American Foresters. Cited in USDA 1995b. .

Gottschalk KW; Thomas SJ; Twardus DB; Ghent JH; Colbert JJ; Teske ME. 1996. GypsES: A Decision support system for gypsy moth management. FRDA Rep. (260): 1-8.

Gottschalk KW; Colbert JJ; Feicht DL. 1998. Tree mortality risk of oak due to gypsy moth. Eur J Forest Pathol. 28(2): 121-132.

Grace JR. 1986. The influence of gypsy moth on the composition and nutrient content of litter fall in a Pennsylvania oak forest. Forest Sci. 32(4): 855-870.

Gribko LS; Liebhold AM; Hohn ME. 1995. Model to predict gypsy moth (Lepidoptera: Lymantriidae) defoliation using kriging and logistic regression. Environ Entomol. 24: 529-537.

Grushecky ST; Liebhold AM; Greer R; Smith RL. 1998. Does forest thinning affect predation on gypsy moth (Lepidoptera: Lymantriidae) larvae and pupae? Environ Entomol. 27: 268-276.

Hajek AE. 1997. Fungal and viral epizootics in gypsy moth populations in central New York. Biol Control. 10(1): 58-68.

Hajek AE. 2001. Larval behavior in *Lymantria dispar* increases risk of fungal infection. Oecologia. 126(2): 285-291.

Hajek AE; Wheeler MM; Eastburn CC; Bauer LS. 2001. Storage of resting spores of the gypsy moth fungal pathogen, *Entomophaga maimaiga*. Biocontrol Sci Technol. 11: 637-647.

Hajek AE; Davis CI; Eastburn CC; Vermeylen FM. 2002. Deposition and germination of conidia of the entomopathogen *Entomophaga maimaiga* infecting larvae of gypsy moth, *Lymantria dispar*. J Invertebr Pathol. 79: 37-43.

Hastings FL; Hain FP; Smith HR; Cook SP; Monahan JF. 2002a. Predation of gypsy moth (Lepidoptera: Lymantriidae) pupae in three ecosystems along the southern edge of infestation. Environ Entomol. 31: 668-675.

Hastings FL; Hain FP; Odell TM. 2002b. A survey of parasitoids and other organisms affecting gypsy moth (Lepidoptera: *Lymantria dispar* L.) along the leading edge of its southward movement. J Entomol Sci. 37: 207-209.

Hellier FF; Warin RP. 1967. Caterpillar dermatitis. Brit Med J. 2: 246-248.

Herrick OW; Gansner DA. 1986. Gypsy moth on a new frontier: forest tree defoliation and mortality. North J Appl Forest. 4:128-133.

Herrick OW; Gansner DA. 1988. Changes in forest condition associated with gypsy moth on new frontiers of infestation. North J Appl Forestry. 5(1):59-61.

Hix DM; Fosbroke DE; Hicks RR Jr; Gottschalk KW. 1991. Development of regeneration following gypsy moth defoliation of Appalachian Plateau and ridge and valley hardwood stands. USDA Forest Service Gen Tech Rep NE-148:347-359. Cited in USDA 1995b.

Houston DR; Valentine HT. 1977. Comparing and predicting forest stand susceptibility to gypsy moth. Can J Forest Res. 7:447-461.

Hutchens JJ Jr; Benfield EF. 2000. Effects of forest defoliation by the gypsy moth on detritus processing in southern Appalachian streams. Am Midl Nat. 143: 397-404.

Johnson WT; Lyon HH. 1988. Insects that feed on trees and shrubs. Cornell University Press. 556 p. Cited in USDA 1995b.

Jones CG; Ostfeld RS; Richard MP; Schauber EM; Wolff JO. 1998. Chain reactions linking acorns to gypsy moth outbreaks and Lyme disease risk. Science. 279: 1023-1026.

Khanislamov MG; Girfanova LN. 1964. Effects of weather conditions on infestations of pests of polyphagous entomophages. In: Biological control of agricultural and forest pests. Natl. Agric. Libr. transl. SB 975 A 333. 164-165. Cited in USDA 1995b.

Kim M.K.; Sisson G.; Stoltz D. 1996. Ichnovirus infection of an established gypsy moth cell line. J Gen Virol. 77: 2321-2328.

Koltunov EV; Andreeva EM. 1999. The abiotic stress as a factor responsible for gypsy moth outbreaks. J Appl Entomol. 123: 633-636.

Kosola KR; Kickmann DI; Paul EA; Parry D. 2001. Repeated insect defoliation effects on growth, nitrogen acquisition, carbohydrates, and root demography of poplars. Oecologia. 129: 65-74.

Kosola KR; Dickmann DI; Parry D. 2002. Carbohydrates in individual poplar fine roots: effects of root age and defoliation. Tree Physiol. 22: 741-746.

Lazarevic J; Peric-Mataruga V; Stojkovic B; Tucic N. 2002. Adaptation of the gypsy moth to an unsuitable host plant. Entomol Exp Appl. 102: 75-86.

Leonard DE. 1981. Bioecology of the gypsy moth. In: Doane CC and ML McManus, eds. The gypsy moth: research toward integrated pest. management. USDA Forest Service Tech. Bull. 1584. Cited in USDA 1995b

Liebhold AM. 1992. Are North American populations of gypsy moth (Lepidoptera: Lymantriidae) bimodal? Environ Entomol. 21:221-229.

Liebhold AM; McManus M. 1991. Does larval dispersion cause the expansion of gypsy moth outbreaks? North J Appl For. 8(3):95-98.

Liebhold AM; McManus M. 1999. The evolving use of insecticides in gypsy moth management. J For. 97(3): 20-23.

Liebhold AM; Zhang X; Hohn ME; Elkinton JS; Ticehurst M; Campbell RW. 1991. Geostatistical analysis of gypsy moth (Lepidoptera: Lymantriidae) egg mass populations. Environ Entomol. 20:1407-1417. Cited in USDA 1995b.

Liebhold AM; Simons EE; Soir A; Unger JD. 1993c. Forecasting defoliation caused by the gypsy moth from field measurements. Environ Entomol. 22:26-32.

Liebhold AM; Elmes GA; Halverson JA; Quimby J. 1994. Landscape characterization of forest susceptibility to gypsy moth defoliation. Forest Sci. 40:18-29.

Lindroth RL; Hwang SY; Osier TL. 1999. Phytochemical variation in quaking aspen: effects on gypsy moth susceptibility to nuclear polyhedrosis virus. J Chem Ecol. 25: 1331-1341.

Liscinsky S. 1984. Tree seed production. Pennsylvania Game News. 55:23-25. Cited in USDA 1995b.

Markovic I; Norris DM; Cekic M. 1996. Some chemical bases for gypsy moth, *Lymantria dispar*, larval rejection of green ash, *Fraxinus pennsylvanica*, foliage as food. J Chem Ecol. 22: 2283-2298.

Marshall E. 1981. The summer of the gypsy moth. Science. 213: 991-993.

Masler EP; Kovaleva ES. 1999. Inhibition of larval growth in the gypsy moth (Lepidoptera: Lymantriidae) by venom from the parasitic wasp, *Microbracon hebetor*. J Entomol Sci. 34(4): 435-444.

Mauffette Y; Lechowicz MJ; Jobin L. 1983. Host preferences of the gypsy moth, *Lymantria dispar* (L.), in southern Quebec. Can J For Res. 13:53-60.

McConnell SP. 1988. Effects of gypsy moth defoliation on acorn production and viability, litterfall, and litter layer depth and biomass in north-central Virginia and western Maryland. MS Thesis. 124 p. Virginia Polytechnic Institute, Blacksburg VA. Cited in USDA 1995b.

McGee CE; Bivens DL. 1984. A billion overtopped white oaks - assets or liabilities? South J Appl For. 8:216-220.

McGraw JE; Gottschalk KW; Vavrek MC; Chester AL. 1990. Interactive effects of resource availabilities and defoliation on photosynthesis, growth, and mortality of red oak seedlings. Tree Physiol 7:247-254. Cited in USDA 1995b.

McShea WJ; Rappole JH. 1992. White-tailed deer as keystone species within forest habitats of Virginia. VA J Sci. 43:177-186. Cited in USDA 1995b.

McShea WJ; Schwede G. 1993. Variable acorn crops: responses of white-tailed deer and other mast consumers. J Mamm. 74:999-1006.

Miller JD; Lindsay BE. 1993a. Willingness to pay for a state gypsy moth control program in New Hampshire: a contingent valuation case study. J Econ Entomol. 86(3): 828-837.

Miller JD; Lindsay BE. 1993b. Influences on individual initiative to use gypsy moth control in New Hampshire, USA. Environ Manag. 17(6): 765-772.

Miller DR; Mo K; Wallner HE. 1989. Influence of climate on gypsy moth defoliation in southern New England. Environ Entom. 18: 646-650.

Montgomery ME. 1990. Role of site and insect variables in forecasting defoliation by the gypsy moth. In AD Watt; SR Leather MD Hunter and NAC Kidd. [Eds.] Population dynamics of forest insects. Intercept, Andover, MA. pp. 73-84. Cited in Gribko et al. 1995.

Montgomery ME. 1993. Untitled letter. Gypsy Moth News. 33: 2-3. Cited in USDA 1995b.

Mosher FH. 1915. Food plants of the gypsy moth in America. USDA Bull 250. 39 p. Cited in USDA 1995b.

Muzika RM; Liebhold AM. 1999. Changes in radial increment of host and nonhost tree species with gypsy moth defoliation. Canadian J Forestry Res. 29:1365-1373.

Muzika RM; Liebhold AM. 2000. A critique of silvicultural approaches to managing defoliating insects in North America. Agric For Entomol. 2(2): 97-105.

Naidoo R; Lechowicz MJ. 1999. Radial growth losses in preferred and avoided tree species during gypsy moth outbreaks. Northern J Appl Forestry. 16:11-18.

Naidoo R; Lechowicz MJ. 2001. Effects of gypsy moth on radial growth of deciduous trees. For Sci. 47: 338-348.

National Gypsy Moth Management Group, Inc. 1991. A rash of gypsy moths: Allergic reactions to caterpillars a serious problem. Newsletter (Spring): 3 pp. Cited in USDA 1995a.

Nealis V.G.; Roden P.M.; Ortiz D.A. 1999. Natural Mortality of the Gypsy Moth Along a Gradient of Infestation. Can Entomol. 131(4): 507-519.

Nixon M; McClain W; Donohoe RW. 1975. Effects of hunting and mast crops on a squirrel population. J Wildl Mgmt. 39:1-25.

NRC (National Research Council). 1983. Risk assessment in the Federal government: managing the process. Washington, DC: National Academy Press; 176 p. + app.

O'Dell TM. 1994. [Supplemental unpublished data on Tuthill study given to Patrick Durkin]. March 15, 1994. USDA Forest Service, Radnor, PA.

Oliver CD. 1978. The development of red oak in mixed stands in central New England. Yale School Forestry Environ Studies Bull 91. 63 p. Cited in USDA 1995b.

Ostfeld RS. 2002. Ecological webs involving acorns and Mice: Basic research and its management implications.   Chapter 13 in: Oak forest ecosystems: Ecology and management for wildlife. pp. 196-214. WJ McShea and WM Healy, Eds.   Johns Hopkins University Press, Baltimore, Maryland.

Ostfeld RS; Jones CG; Wolff JO. 1996. Of mice and mast: Ecological connections in eastern deciduous forests. BioScience. 46(5): 323-330. .

Parker J. 1981. Effects of defoliation on oak chemistry. USDA Tech Bull. 1584:219-225. Cited in USDA 1995b.

Perlman F. 1965. Arthropods in respiratory tract allergy: their relationship to allergens in house dust. Acta Allergologica (Copenhagen). 21: 241-253.

Peterson A. 1990. Ecology and management of a timber rattlesnake (Crotalus horridus L.) population in south-central New York State. Proc. 15th Annual Natural Areas Conf. NYS Mus. Bull. 471:255-261. Cited in USDA 1995b.

Petsonk E. 1994. [Telephone conversation with Patrick R. Durkin]. March 7, 1994. National Institute for Occupational Safety and Health, Montgomery, WV.

Picolo R; Terradas J. 1989. Aspects of crown reconstruction and leaf morphology in *Quercus ilex* L. and *Quercus suber* L. after defoliation by *Lymantria dispar* L. Oecologia Plantarum. 10:69-78.

Podgwaite JD. 1979. Diseases of the gypsy moth: How they help to regulate populations. *In*: Gypsy Moth Handbook. USDA Combined Forest Pest Research and Development Program. Agriculture Handbook No. 539. 15 p.

Podgwaite JD. 1981. Natural disease within dense gypsy moth populations. USDA Forest Service Technical Bulletin 1584. 125-132.

Podgwaite JD; Campbell RW. 1970. Disease in natural gypsy moth populations. *In*: 4th Int. Colloq. Insect Pathol., College Park, MD. p. 279-284.

Potter WD; Deng X; Li J; Xu M; Wei Y; Lappas I; Twery MJ; Bennett DJ. 2000. A Web-based Expert System for Gypsy Moth Risk Assessment. Comput Electron Agric. 27: 95-105.

Quimby JW. 1993. Tree mortality following gypsy moth epidemics – 1990. Gypsy Moth News. 33: 8-12. Cited in USDA 1995b.

Randolph SE. 1998. Mighty theories from little acorns grow: Is Lyme disease risk predictable from mast-seeding by oak trees? Trends in Ecology and Evolution. 13(8): 301-303.

Reardon R; Venables L; Roberts A. 1993. The Maryland Integrated Pest Management Gypsy Moth Project: 1983-1987. USDA Forest Service Radnor, PA. 35 p. .

Redman AM; Scriber JM. 2000. Competition between the gypsy moth, *Lymantria dispar*, and the northern tiger swallowtail, *Papilo canadensis*: Interactions mediated by host plant chemistry, pathogens, and parasitoids. Oecologia. 125(2): 218-228.

Sample B.E.; Butler L.; Zivkovich C.; Whitmore R.C.; Reardon R. 1996. Effects of *Bacillus thuringiensis* Berliner Var. kurstaki and Defoliation by the Gypsy Moth. [*Lymantria dispar* (L.) (Lepidoptera: Lymantriidae)] on Native Arthropods in West Virginia. Can Entomol. 128(4): 573-592.

Schmidt JO. 1982. Biochemistry of insect venoms. Ann Rev Entomol. 27: 339-368.

Schweitzer DF. 1988. Element stewardship abstract for *Lymantria dispar* gypsy moth. The Nature Conservancy, Arlington, VA 22209. 37 p. Cited in USDA 1995b.   .

SERA (Syracuse Environmental Research Associates, Inc.). 2001. Preparation of Environmental Documentation and Risk Assessments, SERA MD 2001-01a, draft dated July 2001. Syracuse Environmental Research Associates, Inc., Fayetteville, NY. Available at www.sera-inc.com.

Shama SK; Etkind PH; O'Dell TM. 1982. Gypsy-moth-caterpillar dermatitis. New Eng J Med. 306: 1300-1301.

Sharov AA; Pijanowski BC; Liebhold AM; Gage SH. 1999. What affects the rate of gypsy moth (Lepidoptera: Lymantriidae) spread: Winter temperature or forest susceptibility? Agric For Entomol. 1(1): 37-45.

Sharov AA; Leonard D; Liebhold AM; Roberts EA; Dickerson W. 2002. "Slow the spread": A national program to contain the gypsy moth. J Forestry 100:30-35.

Sheath RG; Burkholder JM; Morison MO; Steinman AD; Vanalstyne KZ. 1986. Effect of tree canopy removal by gypsy moth larvae on the macroalgae of a Rhode Island USA headwater stream. J Phycol. 22:567-570.

Showalter CR; Whitmore RC. 2002. The effect of gypsy moth defoliation on cavity-nesting bird communities. For Sci. 48: 273-281.

Smith HR. 1985. Wildlife and the gypsy moth. Wildl Soc Bull. 13:166-174.

Smith HR; Lautenschlager RA. 1981. Gypsy moth predators. USDA Tech Bull. 1584: 96-124. Cited in USDA 1995b.

Smitley D; Andresen J; Priest R; Mech R; Mccullough D. 1998. Winter mortality of gypsy moth (Lepidoptera: Lymantriidae) eggs in Michigan. Environmental Entomology. 27(3):700-708.

Stephens GR. 1988. Mortality, dieback, and growth of defoliated hemlock and white pine. Northern J Appl For. 5:93-96.

Sullivan TJ. 1982. Pharmacologic modulation of the whealing response to histamine in human skin: identification of doxepin in a potent in vivo inhibitor. J Allergy Clin Immunol. 69: 260-267.

Summerville KS; Crist TO. 2002. Guild designations and testing for effects of gypsy moth (Lepidoptera: Lymantriidae) outbreaks on native lepidopteran communities: A comment on work and McCullough (2000). Environ Entomol. 31: 581-587. .

Thurber DK. 1993. Effects of gypsy moth-caused tree mortality on bird habitat. In: Fosbroke SLC and KW Gottschalk, eds. Proc., USDA Interagency gypsy Moth Research. Forum Gen Tech Rep. Cited in USDA 1995b.

Thurber DK; McClain WR; Whitmore RC. 1994. Indirect effects of gypsy moth defoliation on nest predation. J Wildl Manage. 58: 493-500.

Tigner T. 1992. Gypsy moth impact on Virginia's hardwood forests and forest industry. Virginia Dept. of Forestry, Charlottesville. 35 p. Cited in USDA 1995b.

Tuthill RW; Canada AT; Wilcock K. 1984. An epidemiology study of gypsy moth rash. Am J Pub Health. 74(8): 799-803.

Twery MJ. 1987. Changes in vertical distribution of xylem production in hardwoods defoliated by gypsy moth. Ph.D. Thesis. Yale University, New Haven. 96 p. Cited in USDA 1995b.

Twery MJ. 1991. Effects of defoliation by gypsy moth. USDA Forest Service Gen Tech Rep. NE-146:27-39. Cited in USDA 1995b.

Twery MJ; Gottschalk KW. 1989. Silviculture vs. the gypsy moth: can it help?   In: Healthy forests, healthy world. Proceedings, Nat. Convention of the Society of American. Foresters. SAF Publ 88-0. Cited in USDA 1995b.

Twery MJ; Mason GN.; Wargo PM; Gottschalk KW. 1990. Abundance and distribution of rhizomorphs of *Armillaria spp*. in defoliated mixed oak stands in western Maryland. Can J Forest Res. 20:674-678.

Uitenbroek DG. 1997. Fisher Exact Test. SISA. Available at: http://home.clara.net/sisa/fisher.htm.

USDA (United States Department of Agriculture). 1995a. Gypsy Moth Management in the United States: A Cooperative Approach. Final Environmental. Impact Statement. Appendix F (Human Health Risk Assessment).

USDA (United States Department of Agriculture). 1995b. Gypsy Moth Management in the United States: A Cooperative Approach. Final Environmental. Impact Statement. Appendix G (Ecological Risk Assessment).

USDA Forest Service. 1990. Gypsy moth research and development program. Northeastern Forest Exp Station, USDA Forest Service. 29 p. Cited in USDA 1995b.

USDA Forest Service (USDA/FS). 1992. Gypsy Moth Exotica. 1:1-4. 4 p. Cited in USDA 1995b.

USDA Forest Service. 1994. The gypsy moth in southern and eastern national forests: to treat or not to treat? Region-8 unpublished draft. Cited in USDA 1995b.

U.S. EPA (U.S. Environmental Protection Agency). 2001. Benchmark Dose Software Version 1.3. EPA 600/R-00/014F. Available at: http://cfpub.epa.gov/ncea/cfm/ recordisplay.cfm?deid=20167.

Vaughan MR. 2002. Oak trees, acorns, and bears.   Chapter 15 in: Oak forest ecosystems: Ecology and management for wildlife. pp. 224-240. WJ McShea and WM Healy, Eds. Johns Hopkins University Press, Baltimore, Maryland.

Vaughan MR; Kasbohm JW. 1993. Response of black bears to gypsy moth infestation in Shenandoah National Park, Virginia. In: Fosbroke SLC and KW Gottschalk, eds. Proc., USDA Interagency gypsy Moth Research. Forum Gen Tech Rep. Cited in USDA 1995b.

Wallner WE. 1994. Research planning and accomplishments. Gypsy Moth Exotica. USDA Forest Service, Northeast Forest Exp Station. 13 pp. Cited in USDA 1995b. .

Wallner WE. 1996. Invasive Pests ('biological pollutants') and US Forests: Whose Problem, Who Pays? OEPP/EPPO Bulletin. 26 (1): 167-180.

Wargo PM. 1977. *Armillaria mellea* and *Agrilus bilineatus* and mortality of defoliated oak trees. Forest Sci. 14:485-492.

Wargo PM. 1981a. Defoliation and tree growth. In: Doane CC and ML McManus, eds. The gypsy moth: research toward integrated pest. management. USDA Tech Bull. Cited in USDA 1995b.

Wargo PM. 1981b. Defoliation, dieback, and mortality. In: Doane CC and ML McManus, eds. The gypsy moth: Research toward integrated pest management. USDA Tech Bull. 1584:240-248. Cited in USDA 1995b.

Wargo PM. 1989. Gypsy moth, *Armillaria*, root disease and oak management interactions. Proceedings 22nd Annual Northeastern Forest Insect Work Conference. pp. 24-26. Cited in USDA 1995b.

Webb JR; Cosby BJ; Deviney FA Jr; Eshleman KN; Galloway JN. 1995. Change in the acid-base status of an Appalachian mountain catchment following forest defoliation by the gypsy moth. Water Air Soil Pollut. 85(2): 535-540.

Weseloh RM. 1996a. Developing and Validating a model for predicting gypsy moth (Lepidoptera: Lymantriidae) Defoliation in Connecticut. J Econ Entomol. 89(6): 1546-1555.

Weseloh RM. 1996b. Effect of supplemental foods on foraging behavior of forest ants in Connecticut. Environ Entomol. 25: 848-852.

Weseloh RM. 1998a. Possibility for recent origin of the gypsy moth (Lepidoptera: Lymantriidae) fungal pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) in North America. Environ Entomol. 27(2): 171-177.

Weseloh RM. 1998b. Modeling the influence of forest characteristics and ant (Formicidae: Hymenoptera) predation on dispersal and survival of neonate gypsy moths (Lymantriidae: Lepidoptera). Environ Entomol. 27: 288-296.

Weseloh RM. 1999. *Entomophaga Maimaiga* (Zygomycete: Entomophthorales) Resting Spores and Biological Control of the Gypsy Moth (Lepidoptera: Lymantriidae). Environ Entomol. 28(6): 1162-1171.

Weseloh RM. 2002. Modeling the impact of the fungus *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) on gypsy moth (Lepidoptera: Lymantriidae): incorporating infection by conidia. Environ Entomol. 31: 1071-1084.

Weseloh RM. 2003. Short and long range dispersal in the gypsy moth (Lepidoptera: Lymantriidae) fungal pathogen, *Entomophaga maimaiga* (Zygomycetes: Entomophthorales). Environ Entomol. 32: 111-122.

White JC. 1901. Dermatitis produced by a caterpillar. Boston Med Surg J. 144(24): 599.

Wilder JW; Christie I; Colbert JJ. 1995. Modeling of Two-dimensional Spatial Effects On the Spread of Forest Pests and Their Management. Ecological Modeling. 82(3): 287-298.

Williams T. 1982. Ah, gypsy moths. Audubon. 84(2):14, 18, 20, 22-23. Cited in USDA 1995b.

Williams DW; Fuester RW; Metterhouse, WW; Balaam RJ; Bullock RH; Chianese RJ. 1991. Oak defoliation and population density relationships for the gypsy moth (Lepidoptera: Lymantriidae). J Econ Entomol. 84(5):1508-1514.

Williams SB; Thompson MK; Roschke DJ. 1997. Gypsy Moth Expert System (GypsES) Review.

Williams RS; Norby RJ; Lincoln DE. 2000. Effects of Elevated CO_2 and Temperature-grown Red and Sugar Maple On Gypsy Moth Performance. Glob Chang Biol. 6(6): 685-695.

Wirtz RA. 1980. Occupational allergies to arthropods documentation and prevention. Bull Entomol Soc Am. 26: 356-360.

Wirtz RA. 1984. Allergic and toxic reactions to non-stinging arthropods. Ann. Rev. Entomol. 29: 47-69.

Work TT; McCullough DG. 2000. Lepidopteran communities in two forest ecosystems during the first gypsy moth outbreaks in northern Michigan. Environ Entomol. 29(5): 884-900.

Table 3-1. Individuals with skin responses to the gypsy moth in two communities (data from Tuthill et al. 1984 except as noted)

Factor	Medway	Lunenburg
Average egg masses/acre	32 [b]	3809 [b]
All groups combined	#Responding/#Exposed	
Total with rash during week before infestation	6/557	7/508
Total with rash during first 7 days after larvae emerge	9/557	50/508
P-value for pre- vs post-emergence difference [b]	0.30	8×10^{-10}
Subgroups		
Age 0-12 years	2/84 [c] (2.3%)	13/69 [d] (19%)
Age 13-59 years	7/407 [c] (1.7%)	35/342 [d] (10%)
Age > 59 years	0/66 [c] (0%)	2/97 [d] (2.1%)
Larval contact		
Touched larvae	8.3%	31.4%
Rash where individuals were touched or crawled on by larvae	29.0%	82.0%

[a] O'Dell 1994

[b] Based on Fisher Exact Test. See text for discussion.

[c] No statistically significant difference among age groups.

[d] Response in 0-12 years significantly greater than 13-59 year group (p=0.039) and >59 year group (p=0.000245). Response in 13-59 year group significantly greater than >59 year group (p=0.0048). All comparisons based on Fisher Exact Test. See text for discussion.

Table 3-2: Statistical Analyses of Epidemiology Data from Table 3-1.

Age Group	Back-ground	Potency (proportion responding per egg mass/acre)	*p*-value	Lower 95% Confidence Interval on Egg masses/acre	
				1%	10%
0-12 years	0.022	4.89e-005	0.00041	128	1336
13-59 years	0.016	2.40e-005	<0.0001	304	3185
>59 years	0.00	5.52e-006	0.15	697	>11,427
All Groups Combined	0.013	2.37e-005	<0.0001	327	3432

[a] Dose associated with a given extra risk – i.e., 1% or 10%.

Table 3-3. Adverse human health effects for members of the general public associated with exposure to the gypsy moth

NOAEL:	32	egg masses/acre	
Level of Infestation	Exposure (egg masses/acre)	Hazard Quotient [a]	Upper Limit on Extra Risk [b]
Sparse	50	1.6	1.4%
Moderate	>50-500	>1.6 - 16	>1.4% - 2.5%
Heavy	>500-5000	>16 - 156	>2.5% - 12%
Extreme	>5,000 - 20,000	>156 - 625	>12% - 38%
LOAEL:	1336	egg masses/acre	
Level of Infestation	Exposure (egg masses/acre)	Hazard Quotient [a]	Upper Limit on Extra Risk [b]
Sparse	50	0.04	Same as above
Moderate	>50-500	>0.04 - 0.4	
Heavy	>500-5000	>0.4 - 4	
Extreme	>5,000 - 20,000	>4 - 15	

[a] Calculated as the exposure in egg masses/acre divided by the NOAEL or LOAEL.
[b] Based on the dose-response model summarized in Table 3-2 using data on all groups combined.

Table 4-1: Summary of quantitative risk characterization for forest stands.

	Forest Stands		
	Sensitive Stands	Intermediate Stands	Tolerant Stands
NOAEL	12	20	125
Egg masses/acre	Hazard Quotients [a]		
5	0.4	0.25	0.04
50	4	2.5	0.4
500	40	25	4
5,000	400	250	40
	Percent Defoliation [b]		
5	5.3%	2.8%	1.8%
50	21.0%	11.0%	6.9%
500	46.0%	24.0%	14.0%
5000	83.0%	43.0%	29.0%

[a] Egg mass density divided by NOAEL
[b] Based on dose-response model of Davidson et al. (2001) detailed in Section 4.3.2.

Figure 2-1: Frequency of defoliation by the gypsy moth from 1975 to 2002 (Source: http://www.fs.fed.us/ne/morgantown/4557/gmoth/defoliation/freq75_02.jpg)

Figure 4-1: Relationship of egg mass density to number of larvae per 50 g dry weight of vegetation (Data from Sample et al. 1996).

Figure 4-2: Summary of Exposure-Response Assessment (see text for details)

Appendix M
Risk Comparison

Figure M-1. Ropes were used to climb trees, to treat them for gypsy moths in the 1930s.

Control/Eradication Agents for the Gypsy Moth - Risk Comparison – Final Report

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Submitted to:
Hank Appleton, COTR
Forest Health Protection Staff
USDA Forest Service
Rosslyn Plaza Building C, Room 7129C
1601 North Kent Street
Arlington, VA 22209

Prepared by Patrick Durkin

Submitted by:
Syracuse Environmental Research Associates, Inc.
5100 Highbridge St., 42C
Fayetteville, New York 13066-0950
Telephone: (315) 637-9560
Fax: (315) 637-0445
E-Mail: SERA_INC@msn.com
Home Page: www.sera-inc.com

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

a.i.	active ingredient
Ach	acetylcholine
AChE	acetylcholinesterase
AEL	adverse-effect level
APHIS	Animal and Plant Health Inspection Service
ARS	Agricultural Research Station
BCF	bioconcentration factor
B.t.k.	*Bacillus thuringiensis* var. *kurstaki*
BIU	Billions of international units
bw	body weight
4-CA	4-chloroaniline
ChE	pseudo-cholinesterase
CNS	central nervous system
DFB	diflubenzuron
EC_x	concentration causing X% inhibition of a process
EIS	environmental impact statement
FH	Forest Health
FS	Forest Service
FTU	forestry toxic units
HQ	hazard quotient
IRIS	Integrated Risk Information System
IU	international units
kg	kilogram
L	liter
LdNPV	gypsy moth (*Lymantria dispar*) nucleopolyhedrosis virus
lb	pound
LC_{50}	lethal concentration, 50% mortality
LD_{50}	lethal dose, 50% mortality
LD_{95}	lethal dose, 95% mortality
LOAEL	lowest-observed-adverse-effect level
m	meter
M	male
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
MSDS	material safety data sheet
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level

NRC	National Research Council
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
OTS	Office of Toxic Substances
PIB	polyhedral inclusion body
ppm	parts per million
PVC	polyvinyl chloride
RfD	reference dose
RQ	risk quotients
UF	uncertainty factor
U.S.	United States
U.S. EPA	U.S. Environmental Protection Agency
USDA	United States Department of Agriculture

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

To convert ...	Into ...	Multiply by ...
acres	hectares (ha)	0.4047
acres	square meters (m^2)	4,047
atmospheres	millimeters of mercury	760
centigrade	Fahrenheit	1.8 °C + 32
centimeters	inches	0.3937
cubic meters (m^3)	liters (L)	1,000
Fahrenheit	centigrade	5/9 (°F-32)
feet per second (ft/sec)	miles/hour (mi/hr)	0.6818
gallons (gal)	liters (L)	3.785
gallons per acre (gal/acre)	liters per hectare (L/ha)	9.34
grams (g)	ounces, (oz)	0.03527
grams (g)	pounds, (oz)	0.002205
hectares (ha)	acres	2.471
inches (in)	centimeters (cm)	2.540
kilograms (kg)	ounces, (oz)	35.274
kilograms (kg)	pounds, (lb)	2.2046
kilograms per hectare (hg/ha)	pounds per acre (lb/acre)	0.892
kilometers (km)	miles (mi)	0.6214
liters (L)	cubic centimeters (cm^3)	1,000
liters (L)	gallons (gal)	0.2642
liters (L)	ounces, fluid (oz)	33.814
miles (mi)	kilometers (km)	1.609
miles per hour (mi/hr)	cm/sec	44.70
milligrams (mg)	ounces (oz)	0.000035
meters (m)	feet	3.281
ounces (oz)	grams (g)	28.3495
ounces per acre (oz/acre)	grams per hectare (g/ha)	70.1
ounces per acre (oz/acre)	kilograms per hectare (kg/ha)	0.0701
ounces fluid	cubic centimeters (cm^3)	29.5735
pounds (lb)	grams (g)	453.6
pounds (lb)	kilograms (kg)	0.4536
pounds per acre (lb/acre)	kilograms per hectare (kg/ha)	1.121
pounds per acre (lb/acre)	mg/square meter (mg/m^2)	112.1
pounds per acre (lb/acre)	ug/square centimeter (ug/cm^2)	11.21
pounds per gallon (lb/gal)	grams per liter (g/L)	119.8
square centimeters (cm^2)	square inches (in^2)	0.155
square centimeters (cm^2)	square meters (m^2)	0.0001
square meters (m^2)	square centimeters (cm^2)	10,000
yards	meters	0.9144

Note: All references to pounds and ounces refer to avoirdupois weights unless otherwise specified.

CONVERSION OF SCIENTIFIC NOTATION

Scientific Notation	Decimal Equivalent	Verbal Expression
1×10^{-10}	0.0000000001	One in ten billion
1×10^{-9}	0.000000001	One in one billion
1×10^{-8}	0.00000001	One in one hundred million
1×10^{-7}	0.0000001	One in ten million
1×10^{-6}	0.000001	One in one million
1×10^{-5}	0.00001	One in one hundred thousand
1×10^{-4}	0.0001	One in ten thousand
1×10^{-3}	0.001	One in one thousand
1×10^{-2}	0.01	One in one hundred
1×10^{-1}	0.1	One in ten
1×10^{0}	1	One
1×10^{1}	10	Ten
1×10^{2}	100	One hundred
1×10^{3}	1,000	One thousand
1×10^{4}	10,000	Ten thousand
1×10^{5}	100,000	One hundred thousand
1×10^{6}	1,000,000	One million
1×10^{7}	10,000,000	Ten million
1×10^{8}	100,000,000	One hundred million
1×10^{9}	1,000,000,000	One billion
1×10^{10}	10,000,000,000	Ten billion

EXECUTIVE SUMMARY

OVERVIEW
The current document provides a comparison of the risks posed by the gypsy moth itself to the risks posed by the different control agents as well as a comparison of risks among the various control agents. The agents used in control programs include *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*), the gypsy moth nucleopolyhedrosis virus (LdNPV), diflubenzuron, tebufenozide, DDVP, and disparlure.

The gypsy moth itself poses the clearest risks in both the human health and ecological risk assessments. If the gypsy moth is not controlled, population outbreaks will occur and humans will be exposed to large numbers of gypsy moth larvae. If this occurs, a substantial number of individuals will experience skin irritation that is sufficiently severe to warrant medical attention. No more serious effects are likely. Ecologically, the gypsy moth will clearly damage some terrestrial vegetation and may directly affect some other species of moths. Because of the obvious importance of vegetation to the existence and habitat of most animals, defoliation by the gypsy moth will have numerous secondary effects.

Most of the control agents also pose risks and raise concerns, the nature and certainty of which are highly variable. In applications used to control the gypsy moth, *B.t.k.* is associated with irritant effects in humans; however, the severity of these effects appears to be less than those associated with exposure to the gypsy moth itself. The potential for *B.t.k.* to cause more serious human health effects is considered but appears to be remote. *B.t.k.* may also cause adverse effects in nontarget *Lepidoptera*. Concern for this effect is heightened because some of the *Lepidoptera* that may be adversely affected include at least on endangered species. Diflubenzuron does not appear to present any substantial risk to human health, and this assessment encompasses 4-chloroaniline, a potential carcinogen that is formed in the degradation of diflubenzuron. Diflubenzuron, however, is a rather nonspecific insecticide and is likely to impact both terrestrial and aquatic arthropods. Tebufenozide is a somewhat more specific insecticide but is used at higher application rates that may lead to high exposures in some terrestrial mammals. The likelihood of observing adverse effects, however, is unclear. Tebufenozide may also impact some nontarget moths and butterflies but should not adversely affect any aquatic species. Although DDVP is a broad spectrum insecticide and can be highly toxic to humans, adverse human health and ecological effects are not expected under normal conditions of use. If DDVP is improperly handled, exposures could substantially exceed prudent levels. For disparlure, exposure estimates for aquatic invertebrates approach a level of concern. More significantly, there is substantial uncertainty in the risk characterization of disparlure because of the limited acute toxicity data, the lack of chronic toxicity data, and the high likelihood that many species will be exposed to this compound.

Unlike all of the other agents considered in this risk assessment, there is no basis for asserting that the use of LdNPV to control or eradicate gypsy moth populations is likely to cause any adverse effects in any species other than the gypsy moth.

PROGRAM DESCRIPTION
The USDA control programs for the gypsy moth are intended to limit damage to forests that can be substantially impacted by gypsy moth outbreaks. Two biological agents that are pathogenic to the gypsy moth are used in broadcast applications: *B.t.k.* and LdNPV. In addition, three chemical agents are used in broadcast applications: diflubenzuron, tebufenozide, and disparlure. Diflubenzuron and tebufenozide are both insecticides, and, as discussed in subsequent sections of the this document, are quite similar with respect to their toxicological properties. The major difference between the two is that application rates for tebufenozide are higher than those for diflubenzuron and this is a controlling factor in the comparative risk assessment for these two agents. Disparlure is a gypsy moth pheromone that is used in broadcast applications to disrupt mating and in population monitoring programs to attract the male gypsy moth to sampling traps. In the past, disparlure was used in a slow-release flake formulation. DDVP is not used in broadcast applications and is used only as a PVC formulated product in milk carton traps used in mass trapping operations.

The USDA adopted various intervention strategies roughly categorized as suppression, eradication, and slow-the-spread. Suppression programs have relied predominantly on *B.t.k.* and diflubenzuron. Slow-the-Spread programs rely predominantly on the use of disparlure flakes and secondarily on *B.t.k.* applications. Eradication efforts rely predominantly on *B.t.k.* NPV is used in all three strategies but is used on very few acres relative to *B.t.k.*, diflubenzuron, and disparlure flakes.

HUMAN HEALTH RISK ASSESSMENT
Hazard Identification – The gypsy moth, *B.t.k.*, and LdNPV are similar not only because they are biological agents but also because the primary effect associated with each agent is irritation. The gypsy moth causes more pronounced and severe irritation relative to either *B.t.k.* or LdNPV. Of the chemical agents used in gypsy moth control programs, diflubenzuron and tebufenozide are similar to each other in that both cause adverse effects on blood. The risk assessment of diflubenzuron is somewhat more involved than that of tebufenozide because diflubenzuron is degraded to 4-chloroaniline, a compound that is classified as a carcinogen. DDVP and disparlure, the other two chemicals used in gypsy moth control programs, have toxicologic profiles that are very different from each other as well as diflubenzuron or tebufenozide. DDVP is a a well-characterized neurotoxin which was studied extensively in mammals. Disparlure, an insect attractant, was not tested extensively for toxicological effects in mammals.

Exposure Assessment – The exposure assessments of the biological agents differ substantially from those of the chemical agents in terms of how the exposures are expressed. Because of the available exposure and toxicity data, different measures of exposure are used for each of the biological agents – i.e., the gypsy moth, *B.t.k.*, and LdNPV. For the chemical agents, all exposure assessments are based on the amount or concentration of the chemical to which an individual or population might be exposed via ingestion, dermal contact, or inhalation. Differences among the chemical agents are dictated largely by differences in how the chemicals are used and, to a lesser extent, on the available toxicity data.

A very different set of exposure assessments is conducted for each of the biological agents. Both *B.t.k.* and LdNPV may also be applied in broadcast applications and the routes of plausible exposure are the same as those for the chemicals applied in broadcast applications – i.e., oral, dermal, and inhalation. For *B.t.k.*, however, the most directly relevant data used to characterize risk are based on actual applications of *B.t.k.* formulations where exposure is best characterized as an application rate. For the assessment of the potential for serious adverse effects, exposures are measured in colony forming units (cfu). LdNPV differs from all of the other agents in that no clear hazard potential can be identified; consequently, the most meaningful measure of exposure is, in some respects, moot. Those exposures that are quantified in the human health risk assessment for LdNPV are based on the mass of the formulation, Gypchek. Exposures to the gypsy moth itself are based on an indirect measure of exposure, egg masses/acre, because this is the expression of exposure that is used in the dose response assessment.

Differences in the exposure assessments among the chemicals used in USDA programs primarily reflect differences in how the chemicals are applied, what routes of exposure are most substantial, and the nature of the toxicity data. Diflubenzuron, tebufenozide, and disparlure may be applied in aerial broadcast applications and multiple routes of exposure (oral, dermal, and inhalation) are plausible. No chronic exposures for disparlure are conducted, however, because no chronic toxicity data are available on this chemical. DDVP, on the other hand, is used only in milk carton traps and exposures will be minimal under normal conditions, although much higher exposures are possible if the traps are not assembled properly or if individuals tamper with the traps.

Dose-Response Assessment – Dose-response assessments are typically based on an RfD, an estimate of a dose or exposure that is not likely to induce substantial adverse effects in humans. The RfD, in turn, is typically based on a NOAEL (no observed adverse effect level) divided by an uncertainty factor. Risk is then characterized as a hazard quotient (HQ), which is the estimated level of exposure divided by the RfD. If the HQ is below unity—i.e., the exposure is less than the RfD —there is no credible risk. If the HQ is above unity, risk is characterized based on dose-response or dose-severity relationships. The quality of the dose-response assessment depends on the quality of the individual studies, the relevance of the studies to potential human exposures, and the strength of the dose-response relationship.

As in the exposure assessments, the dose-response assessments for the biological agents differ substantially from each other as well as from those of the chemical agents. The dose-response assessment for the gypsy moth itself is based on only a single study; however, the study involves two human populations and demonstrates a clear dose-response relationship. Thus, confidence in the dose-response assessment is high. Two endpoints are considered for *B.t.k.*, irritant effects and more serious toxic effects. While the irritant effects are well documented, there is no apparent dose-response relationship and confidence in the dose-response assessment is classified as medium. The dose-response assessment for more serious effects is based on a single study on mice involving intratracheal exposures. While a clear dose-response relationship is apparent, confidence in the dose-response assessment is low because intratracheal exposures have marginal

(if any) relevance to human exposures, the response was not independently replicated, and the observed response might be an artifact. For LdNPV, no endpoint of concern can be identified. Although the individual studies conducted on LdNPV are somewhat dated, the weight of evidence for LdNPV as well as other similar viruses clearly indicates that no systemic effects in humans are anticipated. Thus, confidence in the dose-response assessment for LdNPV is classified as high.

Following standard practices in USDA risk assessments, risk assessment values available from U.S. EPA are adopted directly unless there is a compelling basis for doing otherwise. This approach is taken because the U.S. EPA will typically devote substantial resources and expertise to the development of risk assessment values and it is not feasible to duplicate this effort in risk assessments prepared for the USDA. In addition, the U.S. EPA has the legislative mandate to develop risk values for pesticides and it is sensible for the USDA to administratively defer to U.S. EPA in this area. When risk values are not available from U.S. EPA, the methods used by U.S. EPA are employed to derive surrogate values. Except for disparlure, chronic RfD values are available from U.S. EPA and these values are used directly. For 4-chloroaniline, the U.S. EPA also derived a cancer potency factor as well as a chronic RfD and these values are used directly in the risk assessment. For DDVP, the U.S. EPA derived an acute RfD, and this value is also adopted in the current risk assessment. A complication with DDVP, however, is that this agent is contained within a PVC strip, which substantially impacts the bioavailability of DDVP. In order to consider this detail quantitatively, a single and somewhat marginal study on the toxicity of DDVP in a PVC strip is used, and confidence in this dose-response assessment is, in turn, marginal. Unlike all of the other chemicals considered in this comparative risk assessment, very little toxicity data are available on disparlure. The U.S. EPA did not derive an RfD for this chemical, and the toxicity data available on disparlure are insufficient to derive a surrogate RfD. Thus, confidence in the dose-response assessment for disparlure is marginal.

Risk Characterization – Of the agents considered in this risk assessment, the gypsy moth and DDVP are clearly agents of marked concern, although the nature of the concerns is different. If the gypsy moth is not controlled, population outbreaks will occur and humans will be exposed to large numbers of gypsy moth larvae. If this occurs, a substantial number of individuals will experience irritant effects that are sufficiently severe to cause these individuals to seek medical attention. No more serious effects are likely. For DDVP, the potential for risk is clear but the likelihood of observing risk seems to be remote. Under normal conditions and proper handling, levels of exposure to DDVP will be negligible and risk will be inconsequential. Workers who mishandle a DDVP-PVC strip and/or members of the general public who handle a DDVP-PVC strip may be exposed to levels of DDVP that are far above acceptable levels. While such exposures are clearly to be avoided, they are not likely to cause frank signs of toxicity. This conclusion is consistent with human experience in the use of DDVP resin strips.

Diflubenzuron and tebufenozide are agents of marginal concern. Under most foreseeable conditions of exposure—i.e., exposure scenarios that might be characterized as typical—exposure levels will be far below levels of concern. At the upper ranges of plausible

exposure – levels that might be characterized as extreme— the hazard quotients for diflubenzuron approach a level of concern (HQs between 0.1 and 0.5 for both diflubenzuron and its 4-chloroaniline metabolite). For tebufenozide, the highest hazard quotient is 1.5, which is characterized as undesirable; however, exposure is not likely to cause overt signs of toxicity. The somewhat higher hazard quotients for tebufenozide, compared with those of diflubenzuron, are due solely to higher application rates.

Among the agents of minimal concern, *B.t.k.* is somewhat problematic. Based on the risk for serious adverse effects, there is clearly no cause for concern (the highest HQ is 0.04). As detailed in the dose-response assessment, this lack of concern is reenforced by a very aggressive and protective interpretation of the available toxicity data. Nonetheless, there is some residual concern with irritant effects. These effects are quite plausible in accidental cases of gross over-exposure – e.g., splashing a formulation into the eye. These kinds of concern are minimal and are common to almost all chemical or biological agents. The more troubling concern involves studies of workers and non-workers who report irritant effects, primarily throat irritation. Whether or not these effects should be attributed to the *B.t.k.* exposure is unclear.

The risk characterization for LdNPV and disparlure is unequivocal. Based on the available information, there is no basis for asserting that any serious adverse effects are plausible. Again, various accidental exposures, including splashing the agent into the eyes, could cause transient irritant effects.

ECOLOGICAL RISK ASSESSMENT
Hazard Identification – Unlike the human health risk assessment, in which the potential effects of the biological agents were similar, the ecological effects profile of each of the biological agents considered in this risk assessment are quite distinct. The gypsy moth will primarily affect sensitive trees, and these effects may be substantial. Because of the obvious importance of vegetation to the existence and habitat quality of most animals, a large number of secondary effects may be produced in many other groups of organisms. There is little indication, however, that the gypsy moth will have marked direct effects on groups of organisms other than sensitive plants. LdNPV, on the other hand, is not likely to have any effect on any species other than the gypsy moth. *B.t.k.* is toxic to nontarget *Lepidoptera* as well as the gypsy moth and some other lepidopteran species. There is very little indication that direct effects on other groups of organisms are plausible. Thus, the potential effects of all of the biological agents are considered relatively specific, with LdNPV showing the greatest degree of specificity (only the gypsy moth), followed by the gypsy moth itself (several types of plants) and *B.t.k.* (several types of *Lepidoptera*).

The chemical agents also differ in specificity: disparlure is most specific, tebufenozide is relatively specific to *Lepidoptera*, diflubenzuron is less specific and may affect many arthropods, and DDVP is a nonspecific biocide toxic to many groups of animals, especially arthropods and vertebrates. As a pheromone, disparlure is almost as specific as LdNPV. It will attract the gypsy moth and two other closely related species, the nun moth (*Lymantria monacha*) and the pink

gypsy moth (*Lymantria fumida*). As with the gypsy moth, both of these *Lymantria* species are forest pests, and adverse effects on these species are not a substantial concern for this risk assessment. In addition, the pink gypsy moth is native to Japan and is not found in the United States. A major qualification with the assessment of the specificity of disparlure is that, as in the human health risk assessment, the information on the toxicity of disparlure to nontarget species is very limited. At least in *Daphnia magna*, a commonly used test species in aquatic toxicity studies, the toxicity of disparlure is relatively high. Both diflubenzuron and tebufenozide are clearly toxic to mammals and at least some arthropods. In mammals, both chemicals will cause adverse effects in blood (methemoglobinemia), as detailed in the human health risk assessment. In both terrestrial and aquatic arthropods, both chemicals will interfere with growth and development. Because of differences in the mechanism of action of diflubenzuron and tebufenozide, tebufenozide appears to be somewhat more selective. Effects in birds have been clearly demonstrated for tebufenozide but not for diflubenzuron. While somewhat speculative, it seems plausible to assert that both diflubenzuron and tebufenozide are likely to affect the blood of birds in a way similar to that seen in mammals. In terms of the mechanism of action, DDVP is a general neurotoxin. In all animals that have nervous systems that involve acetylcholinesterase (AChE) and use acetylcholine (ACh) as a neurotransmitter (a substance necessary to make the nerves work properly), DDVP will be toxic, and sufficiently high exposures to DDVP will be lethal. The definition of *sufficiently high*, however, is critical and variable. Although DDVP is not selective mechanistically, differences in sensitivity among species are substantial. For instance, insects are much more sensitive than mammals or other higher organisms to DDVP exposure. This difference in sensitivity is what characterizes DDVP as an effective insecticide that can be used safely.

Exposure Assessment – Diflubenzuron, tebufenozide, LdNPV, and disparlure may be applied in broadcast applications, which means that the potential for exposure is high and, in many cases, unavoidable. Disparlure, in addition to being used in broadcast applications, is used in traps as an attractant. Under those conditions of use, exposure to disparlure will be variable and primarily incidental. Exposures to the gypsy moth itself also vary, depending on the state of the gypsy moth population—i.e., from low level infestation to outbreak conditions.

Some differences between the human health exposure assessment and the ecological exposure assessment, however, are notable. Table 4-2 does not give a measure of exposure for each agent. This is because the measure of exposure will vary both among agents and among the target groups for each agent. For example, exposures to the gypsy moth are measured as egg masses/acre in the human health risk assessment and this is the same measure of exposure used for terrestrial vegetation. As in the human health risk assessment, egg masses/acre are used as the measure of exposure because this is the primary determinant in the dose-response assessment for plants. For all other species, however, effects from the gypsy moth are likely to be secondary rather than primary. Thus, the exposure assessment for these indirectly affected species is based on defoliation – i.e., the result of the dose-response assessment for terrestrial vegetation is used as the exposure assessment for most other groups of organisms.

Other differences in the exposure assessments for nontarget species are mostly superficial. For each of the chemical agents, the mass of the chemical is typically used as the measure of exposure. Depending on the group, the measure of exposure may be expressed as dose (mg agent/kg bw for most terrestrial species), concentration (mg agent/L of water for aquatic species), or simply as application rate (lb agent/acre). This last measure is used primarily when field studies are the basis for the dose-response assessment.

As in the human health risk assessment, different measures of exposure are used for each of the biological agents. For *B.t.k.*, most of the exposures are characterized simply as an application rate in units of BIU/acre. However, colony forming units are used for some of the mammalian exposure scenarios. Also as in the human health risk assessment, no clear hazard potential is identified for LdNPV. The very few exposure scenarios that are quantified in the ecological risk assessment for LdNPV are based on the mass of the formulation, Gypchek.

The level of detail used in the exposure assessments for the different chemicals reflects both differences in use patterns and the nature of the available toxicity data. Full sets of exposure assessments in several groups of animals are developed for diflubenzuron and tebufenozide. As in the human health risk assessment, the exposure assessment for diflubenzuron is elaborated by the consideration of 4-chloroaniline and the exposure assessment for tebufenozide is elaborated by the consideration of multiple applications.

Disparlure, which also may be applied in aerial broadcast applications, has a much more restricted set of exposure scenarios on far fewer groups of organisms. This difference is due completely to the sparse toxicity data available on this compound. In other words, while a very elaborate set of exposure scenarios could be prepared, these scenarios would serve little purpose because they could not be combined with a dose-response assessment to characterize risk. The exposure assessment for DDVP is also restricted but this restriction is due to the very limited exposures that are plausible because DDVP is used only in milk carton traps and exposures for nontarget species will be minimal under normal conditions.

Dose-Response Assessment – In general, confidence in any dose-response relationship is enhanced if a clear dose-response relationship can be demonstrated and both effect and no-effect exposures have been identified. In the case of LdNPV, however, there is simply no indication that LdNPV or the Gypchek formulation will cause toxicity in any nontarget species at any dose level. All of the risk values for LdNPV are based on no-effect concentrations or doses. While additional studies could be conducted at higher doses and while these studies would enhance confidence in the risk assessment, the NOAEL and NOEC values that have been identified are far above any plausible exposures. Thus, while based on limited data in terms of dose-effect characterization, the dose-response assessment for LdNPV is adequate for risk characterization.

For most of the other agents, the dose-response assessments are reasonably good for the species of greatest concern. Dose-response assessments for DDVP are derived only for mammals, fish, and aquatic invertebrates. This limited approach is taken with DDVP because of the limited use

of DDVP in programs to control the gypsy moth. The DDVP is contained in a PVC strip that is placed in a milk carton trap that includes disparlure as an attractant for the gypsy moth. This type of use limits potential exposure to most nontarget species. A formal dose-response assessment is not conducted for terrestrial invertebrates. This is not due to any lack of data. The toxicity of DDVP to insects and many other invertebrates is very well characterized. DDVP is such a potent insecticide that no formal dose-response assessment is needed. Insects and many other species that enter the trap are likely to be killed by exposure to DDVP.

Disparlure is the other agent for which a full set of dose-response assessments is not developed. As discussed in the hazard identification, this is due to the very limited data that are available on the toxicity of disparlure to nontarget species.

Relatively full dose-response assessments on groups of greatest concern are given for the gypsy moth, *B.t.k.*, diflubenzuron and its 4-chloroaniline metabolite, and tebufenozide. For the gypsy moth, the effect of primary concern is damage to vegetation. While data are available on both lethality in trees as well as defoliation, defoliation is used as the sublethal effect of primary concern. A dose-response assessment is also given for nontarget lepidopterans. While effect and no-effect levels can be identified, the significance of this effect is questionable. In terms of direct effects, terrestrial vegetation is the primary target of concern.

The primary nontarget group of concern for *B.t.k.* involves *Lepidoptera*. A relatively rich set of studies is available in which the sensitivities of nontarget *Lepidoptera* as well as some other insects can be quantified reasonably well based on studies involving exposures that encompass the application rates used to control the gypsy moth. Sensitive nontarget lepidoptera include larvae of the endangered Karner blue butterfly as well as several other types of moths.

Similar types of information are available on diflubenzuron and tebufenozide, and dose-response assessments can be made for the species of primary concern. For both chemicals, this includes nontarget *Lepidoptera* and aquatic invertebrates. Other terrestrial arthropods are also considered for diflubenzuron. In addition, because of the standard tests required by U.S. EPA for the registration of most pesticides, adequate toxicity data are available on mammals, birds, and fish. The toxicity data base for diflubenzuron is somewhat more extensive and sensitivities in nontarget organisms are somewhat better defined in both laboratory and field studies than is the case with tebufenozide.

Risk Characterization – Ecological risk assessments involve, at least implicitly, considerations of thousands of different species and relationships among these species and their habitats. Invariably, however, data are available on only a small subset of these species and field studies provide only limited insight into the complex interrelationships and secondary effects among species. Thus, as in the human health risk assessments, ecological risk assessments cannot offer a guarantee of safety. They can and do offer a means to identify whether or not there is a basis for asserting that adverse effects are plausible and what the nature of these effects might be.

Within these limitations, only LdNPV clearly qualifies as an agent of minimal concern. While there are limitations in the available studies on LdNPV, there is simply no basis for asserting that LdNPV will adversely affect any species except the gypsy moth.

Agents of marked concern included the gypsy moth, *B.t.k.*, and diflubenzuron. The types of concern with each of these agents, however, are quite different. For both the gypsy moth and *B.t.k.*, the concerns are narrow. The gypsy moth will clearly damage some terrestrial vegetation. *B.t.k.* is likely to affect sensitive *Lepidoptera*. Concern with the use of diflubenzuron is broader and includes effects on both terrestrial and aquatic invertebrates.

The designation of the gypsy moth as an agent of marked concern is obvious. The effects of gypsy moth larvae on forests are extremely well documented and well understood. In sensitive forest stands—i.e., stands in which oak, birch, and other favored species predominate —gypsy moth larvae can cause substantial defoliation and tree mortality. While some other lepidopteran species also may be directly affected by exposure to the gypsy moth, most of the other effects caused by the gypsy moth will be secondary. Reductions in populations of squirrels, mice, and other mammals which may be sensitive to changes in the availability of acorns are likely and have been well documented. Substantial secondary adverse effects on other groups of animals – i.e., birds, reptiles, and aquatic species – cannot be ruled out but have not been convincingly or consistently demonstrated.

Diflubenzuron is also clearly an agent of marked concern. Exposures to diflubenzuron at application rates used in gypsy moth control programs will adversely affect both terrestrial and aquatic invertebrates that rely on chitin for their exoskeleton. This effect is demonstrated in controlled toxicity studies as well as multiple field studies.

B.t.k. is considered an agent of marked concern because recent studies convincingly demonstrate that adverse effects in nontarget *Lepidoptera* will occur in the applications of *B.t.k.* used to control the gypsy moth. Concern is heightened because some of the *Lepidoptera* that may be adversely affected include at least one endangered species.

Tebufenozide, DDVP, and disparlure are all classified as agents of marginal concern. For tebufenozide, the numeric expressions of risk may be less relevant than a more qualitative assessment. The highest risk is associated with the consumption of contaminated vegetation by a large mammal after two applications at the highest labeled application rate. It is not clear, however, that any frank signs of toxicity would be seen. Risks to nontarget *Lepidoptera* may be of greater concern, but the available data are insufficient to quantify potential risk. Risks to other invertebrates, both terrestrial and aquatic, appear to be insubstantial. DDVP is of marginal concern in that highly localized effects may be expected: nontarget insects entering a milk carton trap or some aquatic invertebrates affected by the accidental contamination of a small body of water with a pest strip. In both cases, the effects would be relatively minor, in terms of the number of organisms affected. Marginal concern for disparlure is associated with the relatively high toxicity of this agent to *Daphnia*. The very limited information on the toxicity of disparlure

argues for a persistent level of vigilance for this agent that may be applied to large areas in broadcast applications.

1. INTRODUCTION

The USDA is preparing an update to the 1995 Environmental Impact Statement (EIS) for the Cooperative Gypsy Moth Management Program (USDA 1995). As part of this effort, updated risk assessments were developed on each of the chemical and biological control agents that are used in the USDA programs:—i.e., *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*) (SERA 2004a), the gypsy moth nucleopolyhedrosis virus (LdNPV) (SERA 2004b), diflubenzuron (SERA 2004c), tebufenozide (SERA 2004f), DDVP (SERA 2004e), and disparlure as an active ingredient in materials used to attract the gypsy moth (SERA 2004d). In addition, a separate risk assessment was prepared on the gypsy moth (*Lymantria dispar*) itself.

The current document not only compares the risks posed by the gypsy moth itself with the risks posed by the different control agents, but also compares the risks associated with the various control agents. The risk comparison is structured like the individual risk assessments and includes comparisons of uses (Section 2), potential human health effects (Section 3), and potential ecological effects (Section 4). As in the individual risk assessments, each of the comparative risk assessment sections (Sections 3 and 4) has four major subsections, including an identification of the hazards associated with the agents, an assessment of potential exposure, an assessment of the dose-response relationships, and a characterization of the risks associated with each agent.

Each of the individual risk assessments cited above are complex, detailed, and often very large documents. The risk comparison does not attempt to summarize this information again in detail. Instead, it focuses on discussing the nature and quality of the data that support each step of the risk assessments and the uncertainties and limitations in the conclusions that are reached. Thus, with few exceptions, individual studies are not discussed or referenced in the current document. The exceptions primarily involve relatively recent studies that substantially impact the assessment of risk. Most of these studies involve *B.t.k.* (Herms et al. 1995; Hernandez et al. 1999, 2000; Peacock et al. 1998; Petrie et al. 2003).

2. PROGRAM DESCRIPTION

2.1. Overview

The USDA control programs for the gypsy moth are intended to limit damage to forests that can be substantially impacted by gypsy moth outbreaks. Two biological agents that are pathogenic to the gypsy moth are used in broadcast applications: *B.t.k.* and LdNPV. In addition, three chemical agents are used in broadcast applications: diflubenzuron, tebufenozide, and disparlure. Diflubenzuron and tebufenozide are both insecticides and, as discussed in subsequent sections of the this document, have similar toxicologicalal properties. The major difference between the two is that application rates for tebufenozide are higher than those for diflubenzuron, which is a controlling factor in the comparative risk assessment for these two agents. Disparlure is a gypsy moth pheromone used in broadcast applications to disrupt mating and in population monitoring programs to attract the male gypsy moth to sampling traps. In the past, disparlure was used in a slow-release flake formulation. DDVP is not used in broadcast applications and is used only as a PVC formulated product in milk carton traps used in mass trapping operations.

The USDA adopted various intervention strategies roughly categorized as suppression, eradication, and slow-the-spread. Suppression programs have relied predominantly on *B.t.k.* and diflubenzuron. Slow-the-Spread programs rely predominantly on the use of disparlure flakes and secondarily on *B.t.k.* applications. Eradication efforts rely predominantly on *B.t.k.* NPV is used in all three strategies but is used on very few acres relative to *B.t.k.*, diflubenzuron, and disparlure flakes.

2.2. Control Agents

Gypsy moth is a pest species that can cause substantial damage to some forests. In the eastern United States, most hardwood forests are classified as susceptible to gypsy moth infestation and as many as 12.5 million acres have been defoliated in a single season. The gypsy moth is found throughout much of New England and south to Virginia and west to portions of Wisconsin.

In past years, USDA employed chemical and biological agents in gypsy moth control programs. The biological control agents consist of *B.t.k.* and LdNPV. Both of these biological agents are pathogenic to the gypsy moth. The chemicals that may be used in the control of the gypsy moth include diflubenzuron, tebufenozide, and disparlure. Diflubenzuron and tebufenozide are used as direct insecticidal control agents, similar to the uses of *B.t.k.* and LdNPV. All of these agents are used in broadcast aerial or ground applications.

DDVP and disparlure are used in mass trapping. Disparlure attracts the male gypsy moth to a large milk carton trap and the DDVP kills insects that enter the trap. While DDVP functions as an insecticide in the trap, it is not considered a control agent for the gypsy moth because mass trapping is used only in population surveys. Disparlure, in a flake formulation, is also used in broadcast aerial applications. While the disparlure does not cause any direct toxic effects to the gypsy moth, the mass application of disparlure will impair the ability of the male gypsy moth to

find female gypsy moths and thus will limit the ability of gypsy moth populations to propagate. Thus, disparlure is used as a control agent.

All of the agents used in gypsy moth control programs are applied in various types of formulations—i.e., the active ingredient combined with various other chemicals or materials. To the extent possible, these materials are discussed in each of the individual risk assessments. Specific information on inerts, however, is classified as CBI (confidential business Information) under Section 7(d) and Section (10) of FIFRA, and this information cannot be specifically disclosed in a risk assessment. In terms of a comparative risk assessment, however, the most important distinctions involve the formulations of *B.t.k.* and LdNPV in complex mixtures and the use of DDVP in polyvinyl chloride (PVC) strips.

B.t.k. and LdNPV are both applied as very complex mixtures that are not fully or clearly defined. *B.t.k.* is cultured or grown in a medium containing water and nutrients, including sugars, starches, proteins, and amino acids. The nutrients, which are, themselves, chemically complex consist of variable biological materials, including animal foodstuffs, various flours, yeasts, and molasses. Similarly, LdNPV is prepared by isolating the virus from infected gypsy moth larvae. The active material consists of the virus, gypsy moth parts, and residual materials used to isolate and purify the virus. Complex mixtures can pose substantial difficulties in a risk assessment; however, the data on *B.t.k.* and LdNPV involve adequate studies on the toxicity of the complex mixtures. This is particularly true for *B.t.k.* in which much of the information on risk is based on applications of commercial formulations in the field.

DDVP is used only in a PVC strip. Each strip contains 590 mg of DDVP and 89.25% inerts, which consist primarily of the PVC in the strip and plasticizers. The limited use of DDVP and its containment in the PVC strip have a major impact on the risk posed by DDVP, relative to the other compounds used in gypsy moth control programs. This impact is discussed at some length in the DDVP risk assessment and in subsequent sections of this document.

2.3. Application Rates

Application rates for the different control agents differ substantially both in magnitude and, for the biological agents, in the manner in which the application rate is expressed.

For *B.t.k.*, application rates are expressed in billions of international units (BIU), which is a measure of the activity or potency of the formulation rather than an expression of mass. The range of application rates used in USDA programs is 20-40 BIU/acre. For LdNPV, the recommended application rate is 0.43 oz Gypchek/acre for suppression and 1.08 oz Gypchek/acre for eradication. The application rate of 0.43 oz/acre corresponds to about 4×10^{11} PIB (polyhedral inclusion bodies)/acre and the application rate of 1.08 oz/acre corresponds to about 1×10^{12} PIB/acre.

Broadcast application rates are expressed in units of lb a.i./acre. For diflubenzuron, the range of labeled application rates is 0.0078-0.0624 lbs a.i./acre. For tebufenozide, higher labeled

application rates are permitted: 0.03-0.12 lbs/acre. Multiple applications of tebufenozide are also permitted, and the maximum annual application rate is 0.24 lb a.i./acre. The application rates for tebufenozide may vary among USDA programs—i.e., suppression, eradication, and slow-the-spread. For the tebufenozide risk assessment, a range of application rates—i.e., 0.015- 0.12 lb a.i./acre—are considered. All exposure assessments are conducted at the maximum application rate of 0.12 lb/acre, assuming that two applications are made with three days between applications. This worse-case scenario involves the use of two applications that reach the maximum annual application rate of 0.24 lb/acre and the shortest interval between applications. As noted in Section 3.4, the higher application rates for tebufenozide, compared with application rates for diflubenzuron, are the determining factor in the risk comparison. The application rate for disparlure is about 0.064 lb a.i./acre, near the maximum application rate allowed for diflubenzuron. Disparlure, however, is always applied in a slow-release formulation, either flakes or microspheres. DDVP is not applied in broadcast applications. Accordingly, the application rate is not a meaningful measure of exposure for this agent.

2.4. Use Statistics

In order to minimize the ecological effects and human health effects of gypsy moth infestations, the USDA adopted various intervention strategies roughly categorized as suppression, eradication, and slow-the-spread (Liebhold and McManus 1999). Suppression efforts are conducted by the USDA Forest Service in areas of well established gypsy moth infestations to combat or interdict periodic gypsy moth population outbreaks. Eradication efforts are conducted by USDA/APHIS to eliminate gypsy moth populations in areas where new populations of the gypsy moth are found. Slow-the-Spread, as the name implies, is a program to reduce the expansion of gypsy moth populations from areas of established populations to adjacent non-infested areas.

The use of the various control agents in USDA programs is summarized in Table 2-1. This table gives the total number of acres treated with each of the control agents between 1995 and 2003. Suppression programs rely predominantly on *B.t.k.* and diflubenzuron. Slow-the-Spread programs rely predominantly on the use of disparlure flakes and secondarily on *B.t.k.* applications. Eradication efforts rely predominantly on *B.t.k.* NPV is used in all three strategies but is used on very few acres relative to *B.t.k.*, diflubenzuron, and disparlure flakes. As discussed in the risk assessment on NPV, the production of Gypchek is very expensive and the application of this agent is currently limited to areas that are considered environmentally sensitive. As noted above, tebufenozide is not used in gypsy moth programs but may be used in the future. Given the similarities between tebufenozide and diflubenzuron, the use of tebufenozide is likely to be similar to that of diflubenzuron—i.e., primarily in suppression programs.

3. HUMAN HEALTH RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION
3.1.1. Overview
An overview of the comparative hazard identification for the gypsy moth and the agents used in USDA programs to control the gypsy moth is given in Table 3-1. The gypsy moth, *B.t.k.*, and LdNPV are similar not only because they are biological agents but also because the primary effect associated with each agent is irritation. The gypsy moth causes more pronounced and severe irritation relative to either *B.t.k.* or LdNPV. Of the chemical agents used in gypsy moth control programs, diflubenzuron and tebufenozide are similar to each other in that both cause adverse effects on blood. The risk assessment of diflubenzuron is somewhat more involved than that of tebufenozide because diflubenzuron is degraded to 4-chloroaniline, a compound that is classified as a carcinogen. DDVP and disparlure, the other two chemicals used in gypsy moth control programs, have toxicological profiles that are very different from each other as well as from diflubenzuron or tebufenozide. DDVP is a well-characterized neurotoxin and the toxicity of DDVP in mammals has been studied extensively. Disparlure is an insect attractant that has not been extensively tested for toxicological effects in mammals.

3.1.2. Biological Agents
The biological agents—i.e., *B.t.k.*, LdNPV, and the gypsy moth itself—present similar toxicological profiles. All three agents are irritants and cause similar irritant effects. The most likely effect from exposure to the gypsy moth is skin irritation. Gypsy moth larvae, as well as the larvae of many species of *Lepidoptera*, cause skin irritation in humans. The skin reactions seem to be associated with contact with small fine hairs that stick out from the body of the larva. Other effects associated with exposure to gypsy moth larvae include eye and respiratory irritation; however, these effects are not as well documented as the dermal effects.

LdNPV also causes irritant effects. It is likely that the irritant effects are due at least in part to the presence of body parts of gypsy moth larvae in LdNPV preparations. Based on the available animal data, there is clear evidence that Gypchek, the commercial formulation of LdNPV, can cause eye irritation. There is little indication, however, that Gypchek is likely to cause dermal or respiratory irritation, which may have something to do with the processing of the gypsy moth parts during the preparation of Gypchek.

The irritant effects of *B.t.k.* are probably due to the formulation of the bacteria rather than the bacteria itself. As noted in Section 2, commercial preparations of *B.t.k.* are very complex mixtures of the bacteria, fermentation byproducts, and adjuvants. *B.t.k.* formulations, however, are not strong irritants to either the eyes or the skin, except in the cases of accidental and gross contamination of the eyes. Instead, the most consistent effect appears to be irritation of the respiratory tract, particularly the throat.

The irritant effects of the gypsy moth appear to be notably more severe than those of *B.t.k.* The wheals and rashes that result from exposure to the gypsy can cause severe itching which may

persist from several days to two weeks. Moreover, these effects can be severe enough to cause the affected individual to seek medical treatment. The relatively consistent set of epidemiology studies following *B.t.k.* applications note a very different outcome. Despite many reports of irritant effects among exposed individuals, there is not a corresponding increase in the incidence of individuals seeking medical care. Thus, unlike the case in severe gypsy moth infestations, the severity of the irritant effects does not appear to be severe enough for individuals to seek medical care.

There is very little indication that these biological agents will be associated with other more serious effects. LdNPV and Gypchek formulations of LdNPV were tested in relatively standard toxicity studies as well as in pathogenicity studies with no indication of serious effects even at very high doses. The gypsy moth has not been formally tested in human or animal studies; on the other hand, this species has infested North America for more than 100 years and no cases of frank adverse effects associated with gypsy moth exposure are to be found in the available literature. Hence, there appears to be no risk of serious adverse effects from exposure to LdNPV, Gypchek, or the gypsy moth itself.

The potential for *B.t.k.* to produce serious adverse effects is somewhat more complicated than the assessment of LdNPV and the gypsy moth. As discussed in the *B.t.k.* risk assessment, severe adverse effects associated with exposure to *B.t.k.* are not reported in any of several epidemiology studies or standard animal toxicity studies on *B.t.k.* or formulations of *B.t.k.* A recent study by Hernandez et al. (2000), however, reports mortality in mice after intranasal instillations of *B.t.k.* Intranasal instillations of bacteria are analogous to inhalation exposures in that the bacteria are inhaled and transported to the lungs during the course of the study. This route of exposure is used to screen qualitatively for potential toxic effects, particularly for biological agents, and is not commonly used in a quantitative risk assessment because of uncertainties in extrapolating from intranasal doses to inhalation exposures that may occur in humans. In the *B.t.k.* risk assessment, some very conservative assumptions are made in the application of the Hernandez et al. (2000) study to provide an estimate of risk. As with LdNPV and the gypsy moth, this analysis (considered further in Sections 3.3 and 3.4) suggests that the risk of adverse effects is likely to be very low under foreseeable conditions of exposure.

The Hernandez et al. (2000) study also reports that pre-treatment of mice with an influenza virus substantially increased mortality in mice exposed to various doses of *B.t.k.*, again by intranasal instillation. This effect raises concern about the susceptibility of individuals who have influenza or other viral respiratory infections to severe adverse responses to *B.t.k.* exposure. The viral enhancement of bacterial infections is not uncommon and the enhancement of *B.t.k.* toxicity by a viral infection is, in some respects, not surprising. The relevance of this observation to public health cannot be assessed well at this time. No such effects are reported in the epidemiology studies conducted to date. It is, however, not clear that the epidemiology studies would detect such an effect or that such an effect is plausible under the anticipated exposure levels (typical or extreme) used in programs to control the gypsy moth. The viral enhancement of *B.t.k.* toxicity is likely to be an area of further study in the coming years.

3.1.3. Chemical Agents

In terms of potential human health effects, diflubenzuron and tebufenozide are similar to one another in that both cause adverse effects on blood. DDVP and disparlure, the other two chemicals used in gypsy moth control programs, have toxicological profiles that are very different from one another as well as from diflubenzuron or tebufenozide. The toxicity of DDVP, which is a well-characterized neurotoxin, has been studied extensively in mammals. Disparlure is an insect attractant that has not been extensively tested for toxicologicalal effects in mammals.

3.1.3.1. Diflubenzuron and Tebufenozide – For both diflubenzuron and tebufenozide, the most sensitive effect in mammals involves damage to hemoglobin, a component in red blood cells that is responsible for transporting oxygen throughout the body. If this function is impaired, either because of damage to hemoglobin or lack of oxygen in the air, serious adverse effects (i.e., equivalent to suffocation) can occur. Both diflubenzuron and tebufenozide cause the formation of methemoglobin, a form of hemoglobin that is not able to transport oxygen. Both chemicals causes other effects on the blood; however, methemoglobinemia is the most sensitive effect—that is, the effect that occurs at the lowest dose.

While effects on the blood are well documented, there is less of an indication that diflubenzuron or tebufenozide will cause other specific forms of toxicity. Neither diflubenzuron nor tebufenozide appears to be carcinogenic, mutagenic, neurotoxic or immunotoxic. Furthermore, these chemicals do not appear to cause birth defects or affect endocrine function in laboratory mammals. Diflubenzuron does not appear to cause reproductive effects. Tebufenozide, on the other hand, is associated with adverse reproductive effects in experimental mammals. These reproductive effects, however, occur at doses higher than those associated with methemoglobinemia. Neither diflubenzuron nor tebufenozide have a high order of acute oral toxicity. Diflubenzuron is relatively nontoxic by oral administration, with reported single-dose LD_{50} values ranging from greater than 4640 to greater than 10,000 mg/kg. Similarly, single oral gavage doses of tebufenozide at 2000 mg/kg caused no observable signs of toxicity in mice or rats.

Diflubenzuron is degraded to 4-chloroaniline in the environment. While most chemicals are metabolized in some way, the formation of 4-chloroaniline from diflubenzuron must be and is explicitly considered in the risk assessment because 4-chloroaniline is classified as a carcinogen. This is the only identified carcinogen associated with any of the agents used to control the gypsy moth.

3.1.3.2. DDVP – DDVP is an organophosphorus insecticide that works by inhibiting cholinesterase. Inhibition of this enzyme in mammalian systems produces a variety of systemic effects, including salivation, urination, lacrimation, convulsions, increased bronchial secretions, respiratory depression, and even death. The nature and magnitude of the toxic effects produced by a given exposure to DDVP by any route are directly related to the dose and rate at which the exposure occurs.

In the case of the USDA programs for the management of the gypsy moth, the use of milk carton traps containing Vaportape II (slow-release of DDVP from PVC strips) precludes rapid exposures to high doses of DDVP. The decrease in toxicity of DDVP in a PVC formulation has been studied directly. For the technical grade liquid DDVP, the acute oral LD_{50} in young pigs is about 160 mg/kg and signs of toxicity in these animals were consistent with the general signs of acetylcholinesterase (AChE) inhibition. In a similar bioassay using a PVC formulation, no deaths occurred at doses up to 1000 mg/kg. This key study on the comparative toxicity of DDVP and DDVP-PVC formulations is discussed further in the dose-response assessment (Section 3.3).

DDVP is a very well studied compound and threshold doses for cholinesterase inhibition are well characterized. Short-term animal studies using technical grade DDVP indicate that oral exposures to doses below about 0.5 mg/kg-day (or inhalation exposures to 1-2 mg/m³) do not result in meaningful reductions in cholinesterase activity. Experiments in laboratory mammals exposed to DDVP during pregnancy (by oral or inhalation route) did not show any effect on fertility or health of the offspring, even at levels that produced maternal toxicity. The latest evaluation of data from assays for carcinogenicity and genetic toxicity classify DDVP as a "suggestive" carcinogen and determined that a quantitative assessment of cancer risk is not applicable. The literature contains some data suggesting that contact dermatitis (as well as cross-sensitization to other pesticides) may occur; although, this appears to be an infrequent occurrence in the general population.

3.1.3.3. Disparlure – In the registration of most pesticides, the U.S. EPA requires a relatively standard set of toxicity data covering multiple routes and durations of exposure as well as a number of specific endpoints of concern (e.g., carcinogenicity, reproductive toxicity, neurotoxicity, etc.). These requirements have been applied to diflubenzuron, tebufenozide, and DDVP but not to disparlure. Because of the apparently low toxicity of most pheromones to mammals and because of the low concentrations that are expected in the environment, U.S. EPA requires less rigorous testing of insect pheromones than is required of insecticides (U.S. EPA 2004).

The prudence of these assumptions may be argued but this issue is beyond the scope of the current risk assessment except to note that the application rate for disparlure is somewhat higher than the application rate for diflubenzuron—i.e., up to 0.0624 lbs a.i./acre for diflubenzuron and about 0.064 lb a.i./acre for disparlure (see Section 2). Nonetheless, as noted in Section 2, disparlure is always applied in a slow-release formulation (either flakes or microspheres) and the limited available monitoring data (Section 3.2), do support the assumption that exposures to disparlure are likely to be very low.

In terms of the hazard identification, the result of the U.S. EPA position and the more general lack of concern with the toxicity of insect pheromones is that the toxicity of disparlure to mammals has not been studied extensively. Except for some standard acute toxicity studies in laboratory mammals, few data are available regarding the biological activity of disparlure in mammals. Results of acute exposure studies for oral, dermal, ocular, and inhalation exposure to

disparlure show no indication of adverse effects. The acute toxicity of disparlure in mammals is very low. The LD_{50} of a single dose administered to rats by gavage exceeds 34,600 mg/kg. No studies investigating the effects of chronic exposure of mammals to disparlure or studies investigating the effects of disparlure on the nervous system, immune system, reproductive system, or endocrine system were identified. The carcinogenic potential of disparlure has not been assessed. In a single study on mutagenicity, there was no indication that disparlure is mutagenic.

A case report of an accidental exposure indicates that disparlure may persist in humans for years. This case report concerns an individual involved in the early testing of disparlure who came into contact with the chemical. For more than 10 years after exposure to disparlure, the individual tended to attract male gypsy moths. This nuisance effect is the only well documented result of exposures to disparlure that might occur in USDA programs.

3.2. EXPOSURE ASSESSMENT
3.2.1. Overview
A summary of the exposure assessments for each of the agents covered in the risk assessment is given in Table 3-2. The exposure assessments of the biological agents differ substantially from those of the chemical agents in terms of how the exposures are expressed. Different measures of exposure are used for each of the biological agents—i.e., the gypsy moth, *B.t.k.*, and LdNPV. For the chemical agents, all exposure assessments are based on the amount or concentration of the chemical to which an individual or population might be exposed via ingestion, dermal contact, or inhalation. Differences among the chemical agents are dictated largely by differences in how the chemicals are used and, to a lesser extent, on the available toxicity data.

A very different set of exposure assessments is conducted for each of the biological agents. Both *B.t.k.* and LdNPV may also be applied in broadcast applications and the routes of plausible exposure are the same as those for the chemicals applied in broadcast applications—i.e., oral, dermal, and inhalation. For *B.t.k.*, however, the most directly relevant data used to characterize risk are based on actual applications of *B.t.k.* formulations where exposure is best characterized as an application rate. For the assessment of the potential for serious adverse effects, exposures are measured in colony forming units (cfu). LdNPV differs from all of the other agents in that no clear hazard potential can be identified. Thus, the most meaningful measure of exposure is in some respects moot. Those exposures that are quantified in the human health risk assessment for LdNPV are based on the mass of the formulation, Gypchek. Exposures to the gypsy moth itself are based on an indirect measure of exposure, egg masses/acre, because this is the expression of exposure that is used in the dose-response assessment.

Differences in the exposure assessments among the chemicals used in USDA programs primarily reflect differences in how the chemicals are applied, what routes of exposure are most substantial, and the nature of the available toxicity data. Diflubenzuron, tebufenozide, and disparlure may be applied in aerial broadcast applications that lead to multiple routes of exposure (oral, dermal, and inhalation). No chronic exposures for disparlure are conducted, however, because no chronic toxicity data are available on this chemical. DDVP, on the other hand, is used only in milk carton traps and exposures will be minimal under normal conditions, although much higher exposures are possible if the traps are not assembled properly or if individuals tamper with the traps.

3.2.2. Biological Agents
The exposure assessments for the biological agents —i.e., the gypsy moth, *B.t.k.*, and LdNPV differ substantially from each other, and these differences are largely dictated by the nature of the toxicity data available on each agent and the resulting dose-response assessments (Section 3.3.2).

3.2.2.1. Gypsy Moth – For the gypsy moth, the most direct and relevant measure of human exposure is probably the number of larvae per unit area or tree because it is contact with the larvae that causes skin irritation, the adverse effect typically associated with the gypsy moth. Nonetheless, the available dose-response data are based on studies in which exposure is

quantified as the number of eggs masses/acre. Accordingly, egg masses/acre is the exposure measure used in this risk assessment. As long as gypsy moth populations remain sparse, the larvae usually eat only a small proportion of the foliage of even their most favored host species, and contacts with people are rare. In such cases, egg masses generally do not exceed 50 egg masses/acre. During full-scale outbreaks, densities of about 5000 egg masses/acre are common and densities greater than 20,000 egg masses/acre are occasionally recorded. In such outbreaks, the numbers of gypsy moth larvae can reach up to 50,000 larvae per tree and exposure to the larvae will be essentially unavoidable for individuals near infested trees.

3.2.2.2. B.t.k. – The exposure assessment for *B.t.k.* is unusual in two respects. First, the most directly relevant data used to characterize risk are based on actual applications of *B.t.k.* formulations where exposure is best characterized as an application rate. As summarized in Section 3.3.2, epidemiology studies are available that report responses in populations after applications of *B.t.k.* in the range of those used in USDA programs to control the gypsy moth—i.e., 20-40 BIU/acre. Thus, these studies are used directly in the risk characterization and explicit exposure assessments and dose-response assessments are not needed.

Second, the apparent lack of a specific mechanism of toxicity for *B.t.k.* makes selecting the most appropriate measure of exposure somewhat arbitrary. The potency of *B.t.k.* is often expressed as BIU or FTU and exposures or application rates are expressed in units of BIU or FTU per acre. Although these units may be meaningful expressions of exposure for the gypsy moth, they are not necessarily or even likely to be a meaningful measures of human exposure. Exposure data are available, however, on *colony forming units* or cfu. When *B.t.k.* formulations are applied, either by aerial spray or ground spray, one or more viable spores contained in droplets or particulates is suspended in the air and deposited on sprayed surfaces. These droplets may be collected, either by air sampling or direct deposition, onto various types of filters. The filters are then cultured in a nutrient medium under conditions conducive to bacterial growth. As the bacteria grow, visible masses of bacteria, referred to as colonies, appear on the media. The significance of cfu as a measure of human exposure is limited because there is little indication that *B.t.k.* is a human pathogen. Consequently, the number of viable spores, albeit an important measure of exposure for the gypsy moth, does not appear to be toxicologicalally significant to humans. In this respect, cfu, like BIU, are of limited significance.

Nonetheless, at least for short-term exposures, cfu can be used as a practical measure of relative exposure to a *B.t.k.* formulation. Based on cfu, ground workers may be exposed to much higher concentrations of *B.t.k.* than other groups—i.e., 200,000-15,800,000 cfu/m^3. Much lower exposures, 400-11,000 cfu/m^3, have been measured in workers involved in aerial applications. During spray operations, members of the general public may be exposed to concentrations in the ranging from about 200-4000 cfu/m^3.

3.2.2.3. LdNPV – Given the failure to identify any hazard associated with LdNPV or the Gypchek formulation, there is little need to conduct a detailed exposure assessment for Gypchek. Gypchek contains gypsy moth parts, and these constituents, like the gypsy moth larvae

themselves, cause irritant effects in humans. The use of Gypchek, however, will not substantially increase the overall adverse effects of gypsy moth exposure in infested areas. On the contrary, the use of Gypchek will decrease the potential for human exposure to gypsy moth larvae by reducing larval populations. Based on simple physical processes associated with the application of any pesticide, it is possible to construct any number of exposure scenarios for Gypchek. The risk assessment for LdNPV focuses on one extreme exposure scenario involving the accidental spray of a home garden. While Gypchek is not intentionally applied to such vegetation, the inadvertent spray scenario is plausible. Based on this accidental exposure scenario, the estimated dose to an individual is 0.034 mg Gypchek/kg bw, with an upper range of 0.66 mg Gypchek/kg bw.

3.2.3. Chemical Agents

***3.2.3.1. Diflubenzuron and Tebufenozide* –** Diflubenzuron and tebufenozide are applied in broadcast applications. The available data regarding the toxicity and environmental fate of these chemicals support a standard set of exposure scenarios involving worker exposure (both routine and accidental) and exposures of the general public to direct spray, dermal contact with contaminated vegetation, as well as the acute and longer-term consumption of contaminated food and water. For both of these chemicals, all exposure assessments are conducted at the maximum application rates. For diflubenzuron, all exposure assessments are conducted at the maximum single application rate for diflubenzuron of 0.0625 lb/acre, which is also the maximum amount that can be applied in a single season. The exposure assessments of tebufenozide are somewhat more elaborate because both single and multiple applications must be modeled—i.e., one or two applications at 0.12 lb/acre. While diflubenzuron is modeled at only the single maximum application rate, the exposure assessment for diflubenzuron is made elaborate by the quantitative consideration of the formation of 4-chloroaniline as an environmental metabolite. As noted in Section 3.1, the quantitative consideration of this metabolite is necessary because 4-chloroaniline is classified as a carcinogen and cancer risk is considered quantitatively in the risk characterization.

The exposure patterns for both diflubenzuron and tebufenozide are similar. For workers, three types of application methods are modeled: directed ground spray, broadcast ground spray, and aerial spray. In these general applications, the maximum exposures to workers are similar: 0.009 mg/kg/day for diflubenzuron and 0.02 mg/kg/day for tebufenozide. The differences in worker exposure levels merely reflect the differences in application rates for the two chemicals. Accidental dermal exposures for workers can be much higher: 0.4 mg/kg/day for diflubenzuron and 4 mg/kg/day for tebufenozide. These differences in exposure levels reflect the differences in the concentrations of the two chemicals used in field solutions as well as the differences in the estimated dermal absorption rates.

For members of the general public, the exposure profiles for diflubenzuron and tebufenozide are also similar. The maximum acute exposure levels for both chemicals are associated with contaminated water in an accidental spill scenario: doses of 1.5 mg/kg bw for diflubenzuron and 1.2 mg/kg bw for tebufenozide. Longer-term exposure to both agents, which involves the

consumption of contaminated fruit rather than water, will result in much lower levels of exposure: 0.002 mg/kg/day for diflubenzuron and 0.03 mg/kg/day for tebufenozide. Like workers, members of the general public can be at risk of dermal exposure to diflubenzuron or tebufenozide, and dermal exposure concentrations can be estimated quantitatively. Estimates of dermal exposure, however, are lower than estimates of oral exposure: a maximum of 0.05 mg/kg bw for diflubenzuron and about 0.4 mg/kg bw for tebufenozide.

Exposure assessments for 4-chloroaniline as an environmental metabolite of diflubenzuron are made only for members of the general public. Workers are not considered at risk because significant amounts of 4-chloroaniline are not likely to form during the application of diflubenzuron. For the general public, estimates of exposure to 4-chloroaniline from contaminated vegetation are likely to be about a factor of 50 below the corresponding estimates of exposure to diflubenzuron. The lower estimate of exposure to 4-chloroaniline is due to its expected rapid dissipation from diflubenzuron deposited on vegetation. In water, however, estimated concentrations of 4-chloroaniline are likely to be equal to or greater than anticipated water concentrations of diflubenzuron under certain circumstances. Finally, peak exposures to 4-chloroaniline differ from peak exposures to diflubenzuron in the environment, usually occurring at different times (later after the application of diflubenzuron) and under different conditions of precipitation. These differences are due to the relatively slow rate in the formation of 4-chloroaniline from diflubenzuron in soil.

3.2.3.2. Disparlure – Disparlure is like diflubenzuron and tebufenozide in that all three can be applied by aerial broadcast and multiple routes of acute and longer-term exposure are possible. The exposure assessment for disparlure, however, is much less elaborate than those for diflubenzuron and tebufenozide because of the very limited toxicity data base on disparlure. As discussed in Section 3.3 (Dose-Response Assessment), the U.S. EPA did not derive RfD values for acute or chronic exposure and the available toxicity data do not support the derivation of surrogate values. Thus, in the absence of toxicity data, an elaborate exposure assessment would not be useful in evaluating risk.

For disparlure, dermal exposure is most likely to be the predominant route for occupational exposure and is a possible route of exposure for the general public. A case report involving the accidental exposure of a worker to disparlure indicates that the only notable effect in the worker was the persistent attraction of gypsy moths. Since the available acute systemic toxicity of disparlure in mammals appears to be very low, the absence of dermal absorption data does not add significant uncertainty to this risk assessment. While dermal exposure of workers is expected to be non-toxic, dermal exposure is likely to cause the persistent attraction of gypsy moths.

Both workers and the public may be exposed to disparlure by inhalation, and the magnitude of the exposure can be estimated from available monitoring studies. At application rates more than 15 times the normal application rate (i.e., about 200 g a.i./acre compared with 29.1 g/acre), peak air concentrations ranged from 0.022 to 0.030 $\mu g/m^3$. Adjusted to the normal application rate,

these values correspond to about 0.003-0.004 $\mu g/m^3$, which is far below the air concentration of 5.0 mg/L —equivalent to 5000 µg/L or 5,000,000 µg/m^3—that did not cause mortality or signs of toxicity in experimental animals.

3.2.3.3. DDVP – Unlike the other chemicals used in gypsy moth control programs, DDVP is not applied in broadcast applications. DDVP is used only in a PVC strip that is placed in milk carton traps. Consequently, exposures of both workers and members of the general public should be negligible under normal conditions—i.e., the workers use proper procedures during assembly of the traps and members of the general public do not tamper with the traps. The risk assessment for DDVP does develop exposure scenarios for both workers and members of the general public to encompass improper handling of the DDVP strips by workers or tampering with the traps by members of the general public. These exposures, however, should be considered atypical, and some are extreme.

During assembly, the central estimate of dermal exposures in workers not wearing gloves leads to an absorbed dose of about 0.0008 mg/kg with a range of about 0.0003 mg/kg to 0.004 mg/kg. Inhalation exposures in workers may be highly variable depending on the ventilation rates in an enclosed space and the number of traps that are handled. Based on the handling and transport of 75 traps, inhalation exposures could be as high as 0.6 mg/m^3 in an enclosed and unventilated room and as high as 1.8 mg/m^3 in the passenger compartment of a vehicle. These exposure assessments are based on several site-specific and situation-specific assumptions intended to reflect plausible upper bounds of exposure.

Exposure assessments are also developed for children who might come in contact with an accidentally discarded or misplaced DDVP strip. Estimated dermal doses are much higher than those for workers: a central estimate of about 0.02 mg/kg with a range of 0.003-0.1 mg/kg. Oral exposures from a small child sucking on the pest strip are about a factor of 10 higher than dermal exposures: a central value of about 0.2 mg/kg with a range of 0.04-0.6 mg/kg.

Under normal circumstances, the use of DDVP in PVC strips is not likely to result in contamination of water or other materials that might be consumed by members of the general public. Nonetheless, an exposure assessment is developed for the accidental contamination of a small pond by a pest strip. In this scenario, dose estimates range from about 0.000003 to 0.00007 mg/kg with a central estimate of about 0.00001 mg/kg.

3.3. DOSE-RESPONSE ASSESSMENT
3.3.1. Overview
A summary of the dose-response assessments for each of the agents covered in the risk assessment is given in Table 3-3. Dose-response assessments are typically based on an RfD, an estimate of a dose or exposure that is not likely to induce substantial adverse effects in humans. The RfD, in turn, is typically based on a NOAEL (no-observed-adverse-effect level) divided by an uncertainty factor. Risk is then characterized as a hazard quotient (HQ) which is the estimated level of exposure divided by the RfD. If the HQ is below unity—i.e., the exposure is less than the RfD —there is no credible risk. If the HQ is above unity, risk is characterized based on dose-response or dose-severity relationships. The quality of the dose-response assessment depends on the quality of the individual studies, the relevance of the studies to potential human exposures, and the strength of the dose-response relationship.

As in the exposure assessments (see Section 3.2), the dose-response assessments for the biological agents differ substantially from one another as well as from those of the chemical agents. The dose-response assessment for the gypsy moth itself is based on only one study; however, the study involves two human populations, and a demonstrates a clear dose-response relationship. Thus, confidence in the dose-response assessment is high. Two endpoints are considered for *B.t.k.*, irritant effects and more serious toxic effects. While the irritant effects are well documented, there is no apparent dose-response relationship and confidence in the dose-response is classified as medium. The dose-response assessment for more serious effects is based on a single study in mice that involves intranasal exposures. Although the study demonstrates a clear dose-response relationship, confidence in the dose-response assessment is low because intranasal exposures have marginal (if any) relevance to human exposure, the response was not independently replicated, and the observed response may be an artifact. For LdNPV, no endpoint of concern can be identified. Although the individual studies conducted on LdNPV are all somewhat dated, the weight of evidence for LdNPV and similar viruses clearly indicates the unlikelihood of systemic effects in humans after exposure to LdNPV. Thus, confidence in the dose-response assessment for LdNPV is classified as high.

Following standard practices in USDA risk assessments, risk assessment values available from U.S. EPA are adopted directly unless there is a compelling basis for doing otherwise. This approach is taken because the U.S. EPA will typically devote substantial resources and expertise to the development of risk assessment values and it is not feasible to duplicate this effort in risk assessments prepared for the USDA. In addition, the U.S. EPA has the legislative mandate to develop risk values for pesticides and it is sensible for the USDA to administratively defer to U.S. EPA in this area. When risk values are not available from U.S. EPA, the methods used by U.S. EPA are employed to derive surrogate values. Except for disparlure, chronic RfD values are available from U.S. EPA and these values are used directly. For 4-chloroaniline, the U.S. EPA derived a cancer potency factor as well as a chronic RfD, and these values are used directly in the risk assessment. For DDVP, the U.S. EPA derived an acute RfD, and this value is also adopted in the current risk assessment. A complication with DDVP, however, is that this agent is contained within a PVC strip, which substantially impacts its bioavailability. In order to consider

this matter quantitatively, a single and somewhat marginal study regarding the toxicity of DDVP in a PVC strip is used, and confidence in the dose-response assessment is, in turn, marginal. Unlike all of the other chemicals considered in this comparative risk assessment, very little toxicity data are available on disparlure. The U.S. EPA did not derive an RfD for this chemical, and the available toxicity data are insufficient to derive a surrogate RfD. Thus, confidence in the dose-response assessment for disparlure is marginal.

3.3.2. Biological Agents
3.3.2.1. Gypsy Moth – The dose-response assessment for human health effects is based on reports of skin irritation in two populations: one with low exposure (an average of 32 egg masses/acre) and the other with high exposure (an average of 3809 egg masses/acre). The low-exposure group exhibited no increase in skin irritation. Accordingly, 32 egg masses/acre is taken as a NOAEL (no-observed-adverse-effect level) for humans and is used as a surrogate RfD for exposure to the gypsy moth in a manner analogous to the use of RfD values for control agents. The high exposure group had a significant increase in skin irritation, and, based on a dose-response model developed by U.S. EPA, egg mass densities up to128 egg masses/acre are not likely to cause a detectable increase in skin irritation or rashes.

While the dose-response relationship is based on only two exposure levels, the strength of the dose-response relationship is strong. Typically, an association is judged to be statistically significant if the *p*-value (the probability that the association occurred by chance) is 0.05 or less. For the study on which these dose-response relationships are based, p-values are on the order of 0.0004 or less for most groups. The only exception involves individuals over the age of 59 years. In this group, it is unclear if the lack of a significant response is related to a lesser sensitivity to the gypsy moth or less exposure—i.e., less time spent outdoors.

In addition to these quantitative estimates of response, the severity of the response is important, particularly in a comparison of effects caused by exposure to the gypsy moth and effects caused by exposure to the agents used to control the gypsy moth. Dermal responses to the gypsy moth are sufficiently severe to have generated numerous case reports. While precise statistics are not available, it does appear that the severity of the skin irritation is sufficient to cause appreciable numbers of affected individuals to seek medical care. While exposure to the gypsy moth is associated with irritation to the eyes and respiratory tract, quantitative dose-response relationships for these endpoints cannot be developed.

3.3.2.2. B.t.k. – Two types of dose-response assessments are presented for *B.t.k.*, one for irritant effects and the other systemic toxicity. There is relatively high confidence that formulations of *B.t.k.* will cause various types of irritant effects in humans and experimental animals; however, confidence in the quantitative assessment of these effects is limited by a very weak dose-dependency in the incidence of the response. The quantitative assessment for systemic toxic effects is extremely tenuous because it is based on a very conservative interpretation of a single study using a route of exposure (intratracheal instillation) that typically is not used in quantitative risk assessments.

The estimate for irritant effects is actually a set of observations rather than a formal dose-response assessment. Several epidemiology studies were conducted after *B.t.k.* applications at rates within the range of those used in USDA programs to control the gypsy moth—i.e., 20-40 BIU/acre. Two key epidemiology studies, one involving workers (Cook 1994) and the other involving members of the general public (Petrie et al. 2003), suggest that irritant effects, particularly throat irritation, may be reported in groups of humans during or after applications of *B.t.k.* In the worker study, the data demonstrate a statistically significant increase in the incidence of irritant effects in workers. The significantly increased effects include generalized dermal irritation (dry or itchy skin and chapped lips), irritation to the throat, and respiratory irritation (cough or tightness). Furthermore, the overall incidence of all symptoms combined was increased significantly among the workers, compared with the controls . In the study involving the general public, several types of irritant effects are reported; however, the only effect that is clearly statistically significant involves throat irritation (p=0.002).

Confidence in accepting whether these reports are biologically significant, however, is reduced by the apparent lack of a strong dose-response relationship. The workers were exposed to up to about 16 million cfu/m^3 and the reported incidence of throat irritation is about 24%. In the study involving members of the general public, no measures of exposure are given. Based on monitoring data from similar applications, however, it is likely that members of the general public may have been exposed to air concentrations ranging from approximately 100 to 4000 cfu/m^3 during or shortly after aerial applications of *B.t.k.* This range is a factor of 3950 to 158,000 less than exposures in the worker study. The apparent incidence of throat irritation in the study on members of the general public, however, is about 19%. Thus, while these much lower exposures lead to a somewhat lesser response, the dose-response relationship appears weak. Nonetheless, these studies are taken together to characterize risk semi-quantitatively, as discussed further in Section 3.4.

There is essentially no information indicating that oral, dermal, or inhalation exposure to *B.t.k.* or *B.t.k.* formulations will cause serious adverse health effects. Extremely severe inhalation exposures that coat the test species with commercial formulations of *B.t.k.* are associated with decreased activity, discolored lungs, and other effects but not mortality. Although the animal data are consistent with data regarding human exposure *B.t.k.*, the animal studies are all based on single concentrations and cannot be used in a meaningful dose-response assessment.

Few studies (David 1990; Hernandez et al. 1999,2000) report mortality after exposure to *B.t.k.,* and these studies, while related to inhalation toxicity, involve atypical routes of exposure. One such study (David 1990) was conducted on a *B.t.k.* Dipel formulation after intratracheal instillations. Intratracheal instillations of bacteria are analogous to inhalation exposures in that the bacteria is essentially inserted into the lungs. Toxic responses including death were observed in treated animals, and the time-to-clearance (estimated from linear regression) was prolonged. Hernandez et al. (1999, 2000) assayed the toxicity of *B.t.k.* after intranasal instillations in mice. This method of dosing is also analogous to inhalation exposures in that the material is deposited in nasal passages and the *B.t.k.* is gradually transported to the lungs by inhalation. Doses of 10^2,

10^4, and 10^6 cfu/mouse caused only local inflamation. A dose of 10^8 cfu/mouse resulted in 80% lethality.

In terms of the human health risk assessment, the data from Hernandez et al. (1999, 2000) are not directly useful. Furthermore, the route of exposure (intranasal instillation) makes any use of these data somewhat tenuous.   Concern with the use of this atypical route of exposure in a dose-response assessment is exacerbated because the Hernandez et al. (2000) study does not specify whether or not the instillations were adjusted to a constant volume. If the installations were not adjusted to a constant volume, it is possible that the observed dose-response relationship could be due to differences in volumetric bronchial obstruction or a combination of bronchial obstruction and *B.t.k.*

Notwithstanding these reservations, the Hernandez et al. (1999, 2000) studies provide the best dose-response data available in experimental mammals. Based on a consideration of the Hernandez et al. (2000) study and the estimates of equivalent human exposures, it seems plausible that cumulative exposures up to 1.4×10^{10} cfu/m^3 x hour will not cause adverse effects in humans. This estimate is supported by the worker study (Cook 1994) from which an apparent NOAEL of 3×10^8 cfu/m^3 x hours for adverse health effects in humans can be calculated, and this value is used quantitatively to characterize the potential for serious adverse effects in humans.

3.3.2.3. LdNPV – The dose-response assessment for LdNPV and its formulation as Gypchek is extremely simple, compared with the other biological and chemical agents, except disparlure, used to control the gypsy moth. Due to the lack of systemic toxic effects associated with any plausible route of exposure (i.e., oral, dermal, or inhalation), the U.S. EPA did not derive an acute or chronic RfD for Gypchek. Although this approach is reasonable, the risk assessment for LdNPV, which is used in the EIS, derives a surrogate acute RfD of 26 mg/kg bw. The surrogate RfD, which is based on an experimental acute NOAEL of 2600 mg/kg bw in rats and an uncertainty factor of 100, provides a quantitative basis for comparison between the extremely low risks associated with the application of Gypchek and the risks posed by the other gypsy moth control agents. Confidence in this value is limited because no adverse effect levels were identified—i.e., the true NOAEL for Gypchek may be higher than 2600 mg/kg. This uncertainty in the LdNPV risk assessment is relatively minor, given that even extreme exposures are far below any level of concern (Section 3.4).

Technical grade Gypchek is an eye irritant. While not quantitatively considered in the risk assessment, the distinction between the irritant properties of technical grade Gypchek and the lack of eye irritation associated with Gypchek formulations applied in the field is emphasized in order to highlight areas in which prudent handling practices are likely to be most important.

3.3.3. Chemical Agents
3.3.3.1. Diflubenzuron and Tebufenozide – As discussed in the hazard identification and the exposure assessment, diflubenzuron and tebufenozide are similar to one another in terms of their toxicological profiles. Both chemicals were tested in a similar and relatively standard set of

toxicity studies required by the U.S. EPA for the registration of pesticides. Their most sensitive endpoint, hematological effects (including methemoglobin formation and several other endpoints characteristic of hemolytic anemia) was observed in all mammalian species tested.

Quantitatively, the similarities between diflubenzuron and tebufenozide are further expanded and even more striking in the dose-response assessment. The U.S. EPA derived RfDs for both compounds and the values are virtually identical: 0.02 mg/kg/day for diflubenzuron and 0.018 mg/kg/day for tebufenozide. Even this minor difference is an artifact of rounding. The U.S. EPA agency-wide workgroup, which derived the RfD for diflubenzuron, typically rounds all RfDs to one significant place. The U.S. EPA Office of Pesticides, which derived the RfD for tebufenozide, often reports RfDs to two significant places. If the agency-wide criteria had been applied to tebufenozide, the two RfDs would be identical—i.e., 0.02 mg/kg/day. Since the molecular weights of diflubenzuron (310 g/mole) and tebufenozide (352 g/mole) are so similar, the RfDs would be identical even when expressed in moles—i.e., 7×10^{-5} mMoles/kg/day for diflubenzuron and 5×10^{-5} mMoles/kg/day for tebufenozide.

The RfDs for both chemicals are based on dietary studies in rats, and the respective NOAELs are quite similar: 2 mg/kg/day for diflubenzuron and 1.5-2.4 mg/kg/day for tebufenozide. Again, these minor differences are an artifact of the way in which the dietary concentrations (i.e., mg agent/kg diet) used in the studies were converted to dose estimates expressed as mg/kg bw/day based on food consumption. Both RfDs are also based on an uncertainty factor of 100, a factor of 10 for interspecies differences—i.e., extrapolation of animal data to humans—and a factor of 10 for intraspecies variability—i.e., individuals who might be most sensitive to the chemical. For both chemicals, the U.S. EPA determined that an additional uncertainty factor of 10 for the protection of infants and children, a factor that must be considered under the Food Quality Protection Act (FQPA), is not required. Finally, confidence in both RfDs is high, which is stated explicitly in the Agency wide RfD for diflubenzuron and is implicit in the discussion of the chronic RfD for tebufenozide derived by the U.S. EPA Office of Pesticides—i.e., no data gaps are identified.

The acute dose-response assessments on diflubenzuron and tebufenozide prepared by U.S. EPA are similar in that the U.S. EPA elected not to derive an acute RfD for either compound. This approach is taken because the agency concluded that no endpoint for acute dietary exposure could be identified for either chemical. U.S. EPA identifies an acute NOAEL of 10,000 mg/kg bw for diflubenzuron and an acute oral NOAEL of 2000 mg/kg bw for tebufenozide. For the USDA risk assessments on gypsy moth control agents, surrogate acute RfDs are derived for both chemicals according to the methods typically employed by the U.S. EPA, because many areas of greatest concern involve potential acute effects after accidental or incidental exposures.

For diflubenzuron, a surrogate acute RfD of 100 mg/kg could be derived using the NOAEL of 10,000 mg/kg identified by U.S. EPA. A more conservative approach is taken, however, using the NOAEL of 1118 mg/kg from an acute study (single dose) in which Dimilin 4L, a formulation containing petroleum oil, was used. The resulting surrogate acute RfD is 11 mg/kg. A similar

approach is taken for tebufenozide. Rather than using an acute NOAEL of 2000 mg/kg, a NOAEL of 1000 mg/kg/day in pregnant rats and rabbits, identified by U.S. EPA, is used to derive a surrogate acute RfD of 10 mg/kg/day. Like the chronic RfDs, the acute RfDs are nearly identical.

The dose-response assessment for diflubenzuron is somewhat more complicated than that for tebufenozide because of the need to consider 4-chloroaniline quantitatively. As noted in the hazard identification (see Section 3.1), 4-chloroaniline is an environmental metabolite of diflubenzuron and 4-chloroaniline has been classified as a potential human carcinogen. The U.S. EPA derived a chronic RfD for 4-chloroaniline of 0.004 mg/kg/day, and this value is used to characterize risks from 4-chloroaniline for longer-term exposures. This RfD is based on a chronic oral LOAEL of 12.5 mg/kg/day using an uncertainty factor of 3000, three factors of 10 for interspecies extrapolation, sensitive subgroups, and the use of a LOAEL with an additional factor of 3 due to the lack of reproductive toxicity data. As with diflubenzuron, the U.S. EPA has not derived an acute RfD for 4-chloroaniline. For 4-chloroaniline, a conservative approach is taken in which a surrogate acute RfD of 0.03 mg/kg is based on a subchronic (90-day) NOAEL of 8 mg/kg/day. Consistent with the approach taken by U.S. EPA for the chronic RfD, an uncertainty factor of 300 is used. For cancer risk, the U.S. EPA proposes a human cancer potency factor for 4-chloroaniline of 0.0638 $(mg/kg/day)^{-1}$. This potency factor is used to calculate a dose of 1.6×10^{-5} mg/kg/day that could be associated with a plausible upper limit of cancer risk of 1 in 1 million.

3.3.3.2. Disparlure – As noted in the hazard identification (see Section 3.1.3.3), the U.S. EPA does not require extensive testing of insect pheromones, including disparlure. This approach is taken because insect pheromones are generally regarded as nontoxic to mammals and because these pheromones are commonly employed in very low environmental concentrations. While the merits of this approach may be argued, the result is that there is little information regarding the toxicity of disparlure, and no RfD values, acute or chronic, have been or can be derived.

The only information that can be used to assess the consequences of exposure to disparlure are LD_{50} or LC_{50} values: an oral LD_{50} value greater than 34,600 mg/kg; a dermal LD_{50} value greater than 2025 mg/kg, and an inhalation LC_{50} value greater than 5 mg/L · 1 hour. Notably, each of the values is expressed as "greater than". In other words, less than half of the organisms died at the specified exposure. In the case of disparlure, these values are actually NOEC values for mortality in that none of the animals died during any of the exposures.

3.3.3.3. DDVP – Like diflubenzuron and tebufenozide, and perhaps to an even greater extent, DDVP has an extensive toxicology data base that has been evaluated by numerous government organizations, including U.S. EPA, the Agency for Toxic Substances and Disease Registry (ATSDR), National Institute for Occupational Safety and Health (NIOSH), Occupational Safety and Health Administration, and the World Health Organization. As noted above, these sources are used when possible for selecting levels of acceptable exposure. Because all of the scenarios

considered in this risk assessment involve only acute exposures, only acute exposure criteria are considered.

The acute RfD established by the U.S. EPA for oral and dermal exposure to DDVP, 0.0017 mg/kg, is used for the risk characterization. The RfD is based an acute oral NOAEL of 0.5 mg/kg from a rat study, and the application of an uncertainty factor of 300. Acute exposure criteria proposed by other groups are comparable to but somewhat higher than the acute RfD. Because some of the accidental acute exposures may substantially exceed the acute RfD, some attempt is made to characterize the consequences of high oral exposures. A human NOAEL of 1 mg/kg for AChE inhibition has been identified. While this NOAEL is not used to modify the acute RfD, it can be used to assess plausible consequences of exceeding the RfD. The human data on DDVP, although extensive, are not sufficient to identify a minimal lethal dose. For the current risk assessment, the lowest reported lethal dose (16 mg/kg) is used to assess the plausibility of observing serious adverse effects in cases of accidental overexposure to DDVP.

A number of inhalation criteria are available for DDVP. Since potentially significant inhalation exposures are likely only in workers, the occupational exposure criterion of 0.1 mg/m^3 proposed by American Conference of Governmental Industrial Hygienists is used. This value is a factor of 10 below the occupational criteria proposed by NIOSH and OSHA.

A major factor and a major complication in the dose-response assessment of DDVP involves the formulation of DDVP in a PVC strip. Some of the accidental exposures considered in this risk assessment involve a small child gaining access to a DDVP-PVC strip and being subject to both oral and dermal exposure. While there is little doubt that the PVC strip will slow the rate of exposure and reduce the risk, this is extremely difficult to quantify. Despite the availability of numerous studies regarding the toxicity of DDVP itself, the number of studies regarding the toxicity of DDVP-PVC strips is relatively small. By far the most relevant study is that conducted by Stanton et al. (1979), which clearly indicates that DDVP in a PVC formulation will be much less toxic than unformulated DDVP. The extent of the difference in toxicity can only be semi-quantitatively characterized. For unformulated DDVP, the LD_{50} value was 157 (113–227) mg/kg with no mortality observed at 56 mg/kg. For the DDVP-PVC formulation, no deaths occurred at doses of up to 1000 mg/kg, although signs of toxicity consistent with AChE inhibition were observed at doses of 320 and 1000 mg/kg. Neither tremors nor salivation were observed at doses of 240 or 180 mg/kg. Stanton et al. (1979) do not provide comparative data on the extent of AChE inhibition in unformulated DDVP and the DDVP-PVC formulation.

3.4. RISK CHARACTERIZATION
3.4.1. Overview
Risk characterization is the process of comparing the exposure assessment with the dose-response assessment to express the level of concern regarding a specific exposure scenario or set of scenarios. For systemic toxic effects, risk characterizations are presented as hazard quotients (HQs). A hazard quotient is the ratio of a projected level of exposure divided by some index of an acceptable exposure, such as an RfD. If the HQ is substantially less than one – i.e., the level of exposure is less than the level of acceptable exposure—there is no apparent cause for concern. If the hazard quotient is greater than unity, there is cause for concern.

Because the hazard quotient does not describe dose-response or dose-severity relationships, a comparison of the magnitudes of the hazard quotients among different agents may not be a reliable index of relative risk and other types of information need to be considered. Hazard quotients that are close to a level of concern—i.e., between about 0.1 and 10—may be more difficult to interpret because of uncertainties in both the exposure estimates as well as the dose-response relationships. While the range from 0.1 to 10 is somewhat arbitrary in terms of classifying the nature of concern, this is similar to the approach recently adopted by ATSDR (2004) in which concern for interactions of chemicals is triggered when individual hazard quotients exceed a value of 0.1.

In order to reflect these gradations of concern in the general interpretation of hazard quotients, the comparative risk characterization is not organized by biological and chemical agents (as in the previous sections) but is organized by the nature of the hazard quotients: agents of marked concern (HQ>10), agents of marginal concern (HQ>0.1 but <10), and agents of *minimal* concern (HQ<0.1). The word *minimal* is emphasized because of the inherent limitation in all risk assessments. Risk assessments can never prove absolute safety—i.e., it is impossible to prove the negative, that something does not exist, in this case risk. Risk assessments, however, can be and are used to determine whether or not there is a basis for asserting that risk is plausible.

An overview of the comparative risk characterization is summarized in Table 3-4 and illustrated in Figure 3-1. Of the agents considered in this risk assessment, the gypsy moth and DDVP are clearly agents of marked concern, although the nature of the concerns is different. If the gypsy moth is not controlled, population outbreaks will occur and humans will be exposed to large numbers of gypsy moth larvae. If this occurs, a substantial number of individuals will experience irritant effects that are sufficiently severe to cause these individuals to seek medical attention. No more serious effects are likely. For DDVP, the potential for risk is clear but the likelihood of observing risk seems to be remote. Under normal conditions and proper handling, exposures to DDVP will be negligible and risk will be inconsequential. Workers who mishandle a DDVP-PVC strip or members of the general public who handle a DDVP-PVC may be exposed to levels of DDVP that are far above levels that would be considered acceptable. While such exposures clearly should be avoided, it seems unlikely that they would result in frank signs of toxicity. This conclusion is consistent with human experience in the use of DDVP resin strips.

Diflubenzuron and tebufenozide are agents of marginal concern. Under most foreseeable conditions of exposure—i.e., exposure scenarios that might be characterized as typical—levels of exposure will be far below levels of concern. At the upper ranges of plausible exposure— levels that might be characterized as extreme—the hazard quotients for diflubenzuron approach a level of concern (HQs between 0.1 and 0.5 for both diflubenzuron and its 4-chloroaniline metabolite). For tebufenozide, the highest hazard quotient is 1.5, indicating that, although unlikely to cause overt signs of toxicity, the exposure would be characterized as undesirable. The somewhat higher hazard quotients for tebufenozide are attributed solely to the higher application rates for this compound, compared with diflubenzuron.

Among the agents of minimal concern, *B.t.k.* is somewhat problematic. Based on the risk for serious adverse effects, there is clearly no cause for concern (the highest HQ is 0.04). As discussed in the dose-response assessment, this lack of concern is reenforced by a very aggressive and protective interpretation of the available toxicity data. Nonetheless, there is some residual concern with irritant effects. These effects are quite plausible in accidental cases of gross overexposure—e.g., splashing a formulation into the eye. These kinds of concern are minimal and are common to almost all chemical or biological agents. The more troubling concern involves studies of workers and non-workers who report irritant effects, primarily throat irritation. Whether or not these effects should be attributed to the *B.t.k.* exposure is unclear.

The risk characterization for LdNPV and disparlure is unequivocal. Based on the available information, there is no plausible basis for concern that exposure will cause serious adverse effects. Again, various accidental exposures, such as splashing the agent into the eyes, might cause transient irritant effects.

3.4.2. Agents of Marked Concern
3.4.2.1. Gypsy Moth – Although the quantitative dose-response assessment is based on only one study, the study demonstrates a clear dose-response relationship and is supported by less quantitative reports of irritant effects associated with exposure to the gypsy moth as well as other lepidopteran larvae. In sparse to moderate infestations—i.e., egg mass densities of more than 500 egg masses/acre—adverse effects involving skin irritation are not likely to be detectable in populations of exposed humans. Nonetheless, some individuals who have contact with gypsy moth larvae might develop skin irritation. In heavy gypsy moth infestations—i.e., from more than 500 to 5000 egg masses/acre—the occurrence of adverse skin reactions is expected to be hight, and the effects are likely be severe enough to cause some individuals to seek medical attention. In extreme outbreaks—i.e., greater than 5000 egg masses/acre— the effects will be qualitatively similar to those of severe infestations but may affect up to one-third of the population. Heavy infestations or extreme outbreaks may cause ocular and respiratory effects in some people; nonetheless, there is no way to quantify the likelihood of observing these effects. Similarly, severe infestations are often considered to be a nuisance and cause aesthetic damage to the environment. Both of these factors can lead to stress in some individuals. Young children may be a group at special risk from effects of gypsy moth exposure; however, it is not clear whether children are more sensitive than adults to the effects of gypsy moth exposure or whether

responses in children appear greater because children spend more time outdoors compared with adults.

3.4.2.2. DDVP – In most cases, exposures to both workers and members of the general public should be negligible. If workers take prudent steps to limit both dermal and inhalation exposures, the likelihood of exposures to DDVP reaching a level of concern appears to be very low. Similarly, members of the general public should not be exposed to substantial amounts of DDVP. The DDVP is contained within a PVC strip to ensure that the active ingredient is slowly released over a long period of time. The strip, in turn, is placed within a trap and the trap is placed so that it will not be accessed except in the case of intentional tampering or trap monitoring.

Nonetheless, the risk assessment for DDVP develops exposure scenarios for both workers and members of the general pubic, which are intended to illustrate the potential effects of mishandling or tampering with DDVP strips. For workers, the greatest risks are associated with inhalation exposures from assembling the traps in enclosed and poorly ventilated spaces or transporting the traps in the passenger compartments of vehicles. These risks can be readily avoided. Dermal exposures can also lead to lesser but sill undesirable levels of exposure. For members of the general public, all of the exposure scenarios are accidental and some are extreme. The most likely of these is the accidental contamination of a small body of water. This scenario leads to exposures that are below the level of concern by a factor of about 25. If a child were to come into contact with a DDVP strip, however, both dermal and oral exposures could substantially exceed a level of concern. Although such exposures clearly should be avoided, it seems unlikely that they would result in frank signs of toxicity. This conclusion is consistent with human experience in the use of DDVP resin strips.

3.4.3. Agents of Marginal Concern
3.4.3.1. Diflubenzuron – The risk characterization for potential human health effects associated with the use of diflubenzuron is relatively unambiguous: none of the hazard quotients reach a level of concern at the highest application rate that could be used in USDA programs. In that many of the exposure assessments involve very conservative assumptions—that is, assumptions that tend to overestimate exposure—and because the dose-response assessment is based on similarly protective assumptions, there is no plausible basis for concluding that this use of diflubenzuron poses a hazard to human health.

Notwithstanding the above assertion, it is worth noting that the greatest relative risk concerns the contamination of water with 4-chloroaniline rather than exposure to diflubenzuron itself. The highest hazard quotient for diflubenzuron is 0.1, a factor of 10 below a level of concern. Since this hazard quotient is based on toxicity, an endpoint that is considered to have a population threshold, it is reasonable to assert that the risk associated with exposure to diflubenzuron is essentially zero.

Such is not the case with 4-chloroaniline, which is classified as a probable human carcinogen and is an environmental metabolite of diflubenzuron. For 4-chloroaniline, the highest hazard quotient is 0.4, below the level of concern by a factor of only 2.5. The scenario of greatest concern involves cancer risk from drinking contaminated water. This risk would be most plausible in areas with sandy soil and annual rainfall rates ranging from about 50 to 250 inches. The central estimate of the hazard quotient for the consumption of water contaminated with 4-chloroaniline and based on a cancer risk of 1 in 1million is 0.09, which is 10 times lower than the level of concern.

3.4.3.2. Tebufenozide – The similarities between tebufenozide and diflubenzuron have been emphasized throughout this comparative risk assessment. As noted in the dose-response assessment, the toxicities of these two compounds are virtually identical. While both diflubenzuron and tebufenozide are classified as agents of marginal concern—i.e., risk quotients between 0.1 and 10 —tebufenozide does exceed the level of concern, whereas diflubenzuron does not. This difference is due to the higher application rates that may be used with tebufenozide. These higher application rates for tebufenozide increase the levels of exposure, which results in somewhat higher hazard quotients for tebufenozide, compared with diflubenzuron.

Nonetheless, as with diflubenzuron, there is no clear indication that adverse effects are likely to result from exposure to tebufenozide. At the maximum application rate considered in this risk assessment, two applications at 0.12 lb/acre spaced three days apart, there is little indication that adverse effects on human health are likely and only one scenario exceeds a risk quotient of 1. Based on central estimates of exposure— those that might be considered typical and expected—hazard quotients including workers and members of the general public range from 0.00003 to 0.03, below a level of concern by factors ranging from approximately 30 to 33,000. At the upper range of plausible exposures, the hazard quotient for ground spray workers reaches a level of concern—i.e., a hazard quotient of 1. For members of the general public, the upper range of exposure leads to a hazard quotient of 1.5 for the longer-term consumption of contaminated vegetation following two applications at 0.12 lb/acre. Because of the linear relationship between exposure and application rate, two applications at 0.08 lb/acre would reach but not exceed a level of concern. With a single application at the maximum rate of 0.12 lb/acre, the hazard index is 0.8, below the level of concern. While the longer-term consumption of contaminated vegetation is probably not a likely scenario, it is a standard exposure scenario used in USDA risk assessments to consider the longer-term consumption of food items, like berries, that might be sprayed during the broadcast application of a pesticide. This risk assessment suggests that two applications at 0.08 lb/acre or more should be avoided in areas where members of the general public might consume contaminated fruits or other contaminated vegetation.

3.4.4. Agents of Minimal Concern
3.4.3.1. B.t.k. – The risk characterization regarding exposure to *B.t.k.* and its formulations is generally consistent with that of the previous USDA risk assessment as well as more recent risk assessments conducted by the U.S. EPA and the World Health Organization: *B.t.k.* and its

formulations are likely to cause irritation to the skin, eyes, and respiratory tract; however, serious adverse health effects are implausible. Whether irritation is caused by *B.t.k.* in typical field applications used to control the gypsy moth is uncertain. While epidemiology studies involving self-reporting of symptoms do suggest that reports of irritant effects are to be expected, the biological plausibility of these effects is called into question because of an insubstantial dose-dependency for the irritant effect.

B.t.k. applications to control or eradicate the gypsy moth are not expected to cause serious adverse health effects in humans. At the extreme upper range of exposure in ground workers, exposure levels are estimated to be below the functional human NOAEL for serious effects by a factor of 25. For members of the general public, exposure levels are estimated to be below the functional human NOAEL by factors ranging from about 28,000 to 4,000,000 [4 million]. This assessment is based on reasonably good monitoring data, conservative exposure assumptions, and an aggressive and protective use of the available toxicity data. Based on these data, it is not likely that overt signs of toxicity will be observed in any group— ground workers, aerial workers, or members of the general public—exposed to *B.t.k.* as the result of gypsy moth control and eradication programs conducted by the USDA.

There is no documented evidence of a subgroup of individuals who are more sensitive than most members of the general public to *B.t.k.* formulations. According to a recent epidemiology study, asthmatics are not likely to be affected adversely by aerial applications of *B.t.k.* The literature on *B.t.k.* includes one anecdotal claim of a severe allergy to a carbohydrate in a *B.t.k.* formulation; however, neither the claim nor observations of similar effects are substantiated in the available published epidemiology studies. On the other hand, *B.t.k.* formulations are complex mixtures, and the possibility that individuals may be allergic to some of the components in the formulations is acknowledged by a state health service.

As noted in Section 3.1, pre-treatment with an influenza virus substantially increased mortality in mice exposed to various doses of *B.t.k.* Although the relevance of this observation to public health cannot be assessed well at this time, the viral enhancement of *B.t.k.* toxicity is likely to be an area of further study in the coming years.

3.4.3.2. LdNPV (Gypchek) – There is no plausible basis for concern that either workers or members of the general public are at risk of adverse effects from the use of Gypchek to control the gypsy moth. This statement follows from the failure to identify any hazard associated with exposures to Gypchek or LdNPV and is essentially identical to the risk characterization given by the U.S. EPA.

As discussed in both the exposure and dose-response assessments, the current risk assessment extends the U.S. EPA risk assessment by proposing a surrogate acute RfD and presenting a very conservative exposure assessment based on the accidental spray of a home garden. This approach is taken simply to facilitate the comparison of risks (or lack of risk) associated with Gypchek to the risks associated with other agents used to control the gypsy moth. Based on a

relatively standard dose-response assessment and very conservative exposure assumptions, plausible exposures to Gypchek are below a level of concern by factors ranging from about 50 to more than 750. While more typical exposures—i.e., incidental exposure to Gypchek in water or air—are not provided, they will be substantially less than the range of accidental exposure scenarios used to quantify risk.

3.4.3.3. Disparlure – Although there are studies regarding the acute toxicity of disparlure in laboratory animals, the lack of subchronic and chronic toxicity data precludes a quantitative characterization of risk. The available data regarding the acute toxicity of disparlure indicate that the potential hazard from exposure to the compound is low.

The reliance on acute toxicity data introduces uncertainties into the risk assessment of disparlure that are quite different from the other better studied agents, and these uncertainties cannot be quantified. Other uncertainties in this analysis are associated with the exposure assessment and involve environmental transport and dermal absorption. These uncertainties are relatively minor compared with the lack of subchronic or chronic toxicity data. Thus, while there is no reason to believe that longer-term exposure to disparlure will produce adverse effects, this assumption can not be substantiated due to the lack of chronic toxicity data. The significance of this uncertainty is at least partially offset by the very low exposures that are plausible given the limited use of disparlure. For example, as noted in the dose-response assessment, inhalation exposures of mice to 5 mg/L ($5,000,000 \ \mu g/m^3$) for 1 hour caused no mortality or signs of toxicity. As noted in the exposure assessment, likely concentrations of disparlure in air after applications comparable to those used in programs to control the gypsy moth are likely to be on the order of 0.004 $\mu g/m^3$, a factor of 1,250,000,000 (1.25 billion) below the apparent NOEC for acute toxicity. This relationship is consistent with the general assumption made by the U.S. EPA that exposures to insect pheromones will be far below levels of concern (U.S. EPA 2004).

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview

An overview of the comparative hazard identification is given in Table 4-1. Unlike the human health risk assessment, in which the potential effects of the biological agents are similar, each of the ecological effects profiles of the biological agents considered in this risk assessment is quite distinct. The principal effect of the gypsy moth is damage to sensitive trees, which can be substantial. Because of the obvious importance of vegetation to the existence and habitat of most animals, defoliation by the gypsy moth will have numerous secondary effects in many other groups of organisms. There is, however, no indication that the gypsy moth will have direct effects on groups of organisms other than sensitive plants. LdNPV, on the other hand, is unlikely to have effects on species other than the gypsy moth. *B.t.k.* is toxic to nontarget *Lepidoptera* as well as the gypsy moth and some other lepidopteran species, but is unlikely to have direct effects on other groups of organisms. Thus, the potential effects of all of the biological agents are considered relatively specific, with LdNPV showing the greatest degree of specificity (only the gypsy moth), followed by the gypsy moth itself (several types of plants) and *B.t.k.* (several types of *Lepidoptera*).

The chemical agents also differ in specificity: disparlure is most specific, tebufenozide is relatively specific to *Lepidoptera*, diflubenzuron is less specific and may affect many arthropods, and DDVP is a nonspecific biocide toxic to most groups of animals. As a pheromone, disparlure is almost as specific as LdNPV. It will attract the gypsy moth and two other closely related species, the nun moth (*Lymantria monacha*) and the pink gypsy moth (*Lymantria fumida*). Like the gypsy moth, both of these *Lymantria* species are forest pests, and adverse effects on these species are not a substantial concern for this risk assessment. In addition, the pink gypsy moth is native to Japan and is not found in the United States. A major qualification regarding the specificity of disparlure is the limited amount of information available on nontarget species. The data that are available indicate that the relative toxicity of disparlure to *Daphnia magna*, a commonly used test species in aquatic toxicity studies, is high. Diflubenzuron and tebufenozide are clearly toxic to mammals and at least some arthropods. In mammals, exposure to either chemicals causes adverse effects in blood (methemoglobinemia), as discussed in the human health risk assessment. In terrestrial and aquatic arthropods, exposure to either chemical interferes with growth and development. Because of differences in the mechanism of action of diflubenzuron and tebufenozide, the toxicity of tebufenozide appears to be somewhat more selective. For instance, effects in birds have been clearly demonstrated for tebufenozide but not for diflubenzuron. Nonetheless, it is plausible to speculate that both diflubenzuron and tebufenozide are likely to cause adverse hematological effects in birds, similar to those observed in mammals exposed to these chemicals. In terms of its mechanism of action, DDVP is a general neurotoxin. In all animals that have nervous systems that involve acetylcholinesterase (AChE) and use acetylcholine (ACh) as a neurotransmitter (a substance necessary to make the nerves work properly), DDVP will be toxic and sufficiently high exposures to DDVP will be lethal. The definition of *sufficiently high*, however, is critical and variable. Although DDVP is not selective

mechanistically, differences in sensitivity among species are substantial. Insects are much more sensitive than mammals or other higher organisms to DDVP. This difference in sensitivity is what characterizes DDVP as an effective insecticide that can be used safely.

4.1.2. Biological Agents

4.1.2.1. Gypsy Moth – The clearest primary effect of gypsy moth infestations is on terrestrial plants, primarily trees. Various instars of the gypsy moth larvae will feed on host trees and can cause extensive defoliation which can kill some of the infested trees. On a larger scale, the extensive defoliation and/or death of trees may result in secondary changes to vegetation, which will, in turn, affect other forms of vegetation as well as various animal species (primarily related to changes in habitat). Gypsy moth larvae appear to have definite food preferences; oak, birch, poplar, and apple trees seem to be their favorite food sources. While both the European and Asian gypsy moth cause similar types of damage (i.e., defoliation), their feeding preferences are somewhat different, with the Asian gypsy moth preferring a wider range of vegetation. Heavy defoliation is much more common among the oaks than among trees that are not particularly favored as food by the gypsy moth. For susceptible oaks, the effects of infestations on tree mortality varies according to the initial condition of the stand and the number of infestations. Generally, gypsy moth infestations result in mortality of less than 15% of total basal area—i.e., mortality of trees involving 15% the total area of the tree trunks near the ground. When heavy defoliation is followed by massive overstory mortality, existing shrub and herb cover increase dramatically due to increases in available light, moisture, and nutrients. Extensive loss of the existing canopy will also favor the growth of tree species that are intolerant to shade and will shift the forest ecosystem towards earlier successional stages.

The only other groups of organisms likely to be affected directly by the gypsy moth are some and probably very few other lepidopteran species, including the northern tiger swallowtail butterfly. The mechanisms for direct adverse effects on other lepidopteran species may include bacterial contamination of the leaves by gypsy moth larvae and a decrease in the nutritional value of the leaves damaged by the gypsy moth. Most studies, however, do not indicate substantial direct effects on other insects, including *Lepidoptera*. In some cases, increases may be seen in populations of insect predators of the gypsy moth.

There is no evidence in the literature of direct adverse effects of the gypsy moth on most groups of animals. Indirect effects, associated with damage to vegetation, may be of substantial consequence to some species, including squirrels, mice, and other mammals that rely on acorns. Although some mammals consume insects, including the gypsy moth, there is no evidence that gypsy moth outbreaks have a substantial impact on insectivorous mammals. Similarly, birds and aquatic species are not likely to be affected directly or adversely by the gypsy moth. In some species of birds, gypsy moth infestations and subsequent defoliation may be beneficial, especially for those species favoring dead wood as a habitat.

4.1.2.2. B.t.k. – The hazard identification for mammals is closely related to the hazard identification for the human health risk assessment in that both are based, in part, on numerous

standard toxicity studies in experimental mammals. Although *B.t.k.* may persist in mammals for several weeks after exposure, there is little indication that oral or dermal exposure leads to serious adverse effects. Most inhalation studies do not suggest a potential for adverse effects even at *B.t.k.* concentrations much greater than those likely to be encountered in the environment. The lack of a positive hazard identification is supported by field studies which demonstrate a lack of adverse effects in populations of mammals after applications of *B.t.k.*

Toxicity studies in birds are limited to standard acute exposures required by U.S. EPA for product registration. The studies all involve either single-dose gavage administration or five daily dose gavage administrations, and none of the studies reports signs of toxicity or pathogenicity at single oral doses up to 3333 mg formulation/kg bw or at multiple oral doses up to 2857 mg formulation/kg bw. Due to the lack of toxicity of *B.t.k.* formulations as well as other *B.t.* strains, the U.S. EPA did not require chronic or reproductive toxicity studies in birds. This apparent lack of toxicity is supported by numerous field studies in birds. In one field study, a transient decrease in abundance was noted in one species, the spotted towhee (*Pipilo maculatus*). This observation is inconsistent with other field studies on *B.t.k.,* and, according to the investigators, may be an artifact of the study design.

The mechanism of action of *B.t.k.* in *Lepidoptera* is relatively well characterized. *B.t.k.* vegetative cells produce spores and crystals. After the insect consumes the crystals, toxins are formed that attach to the lining of the mid-gut of the insect and rupture the cell walls. The *B.t.k.* spores germinating in the intestinal tract enter the body cavity through the perforations made by the crystal toxins and replicate causing septicemia and eventually death. While various strains of *B.t.* are often characterized as selective pesticides, *B.t.k.* is toxic to several species of target and nontarget *Lepidoptera*. Sensitive nontarget *Lepidoptera* include larvae of the Karner blue butterfly, two species of swallowtail butterflies, a promethea moth, the cinnabar moth, and various species of Nymphalidae, Lasiocampidae, and Saturniidae.

While some nontarget lepidopteran species appear to be as sensitive as target species to *B.t.k.*, most studies indicate that effects in other terrestrial insects are likely to be of minor significance. There is relatively little information regarding the toxicity of *B.t.k.* or *B.t.k.* formulations to terrestrial invertebrates other than insects. Some oil-based *B.t.k.* formulations may be toxic to some soil invertebrates; however, the toxicity is attributable to the oil in the formulation and not to *B.t.k.* There is no indication that *B.t.k.* adversely affects terrestrial plants or soil microorganisms.

The U.S. EPA classifies *B.t.k.* as virtually non-toxic to fish, and this assessment is consistent with the bulk of experimental studies reporting few adverse effects in fish exposed to *B.t.k.* concentrations that exceed environmental concentrations associated with the use of *B.t.k.* in USDA programs. Although there are no data regarding the toxicity of *B.t.k.* or its formulations to amphibians, other strains of *B.t.* appear to have low toxicity to amphibians. The effects of *B.t.k.* on aquatic invertebrates is examined in standard laboratory studies and in numerous field studies. At concentrations high enough to cause decreases in dissolved oxygen or increased biological

oxygen demand, *B.t.k.* may be lethal to certain aquatic invertebrates, like *Daphnia magna*. Most aquatic invertebrates, however, seem relatively tolerant to *B.t.k.* This assessment is supported by several field studies that have failed to note remarkable effects in most species after exposures that substantially exceed expected environmental concentrations. As with effects on terrestrial plants, the toxicity of *B.t.k.* to aquatic plants has not been tested.

The U.S. EPA's Office of Pesticides (U.S. EPA/OPP 1998) has raised concerns that some batches of *B.t.* may contain heat labile exotoxins that are toxic to *Daphnia*. The production of these toxins is an atypical event thought to be associated with abnormal or poorly controlled production processes. The U.S. EPA requires manufacturers to submit a daphnid study on each new manufacturing process to demonstrate that heat labile exotoxin levels are controlled.

4.1.2.2. LdNPV – Similar to the hazard identification for the human health risk assessment, the hazard identification for nontarget wildlife species fails to identify any adverse effects of concern—i.e., there is no indication that LdNPV or the Gypchek formulation of LdNPV has the potential to cause adverse effects in any nontarget species. The mammalian toxicity data base for LdNPV is reasonably complete and indicates that LdNPV is not pathogenic or otherwise toxic to mammals. One specific study conducted on wildlife mammals that may consume contaminated gypsy moth larvae indicates no adverse effects in mice, shrews, and opossums. Relative to the large number available studies in mammals, few studies are available in birds but the results of these studies are nearly identical to those in mammals indicating that exposures to LdNPV at levels substantially higher than those likely to occur in the environment will not be associated with adverse effects. Based on bioassays of LdNPV on numerous nontarget insect species and supported by the generally high species specificity of related baculoviruses, the hazard identification for LdNPV in nontarget insects is strikingly similar to that in birds and mammals. There is no indication LdNPV will cause adverse effects in nontarget insects at any level of exposure. Relatively few studies regarding the toxicity of LdNPV have been conducted in fish or aquatic invertebrates; nevertheless, these studies are consistent with studies in terrestrial species, indicating a lack of toxicity to fish and aquatic invertebrates. No data are available on the effects of LdNPV on amphibians, aquatic or terrestrial plants, or other microorganisms. While this lack of information does, by definition, add uncertainty to this risk assessment, there is no basis for asserting that effects on these or other organisms are plausible.

4.1.3. Chemical Agents
4.1.3.1. Diflubenzuron and Tebufenozide – The toxicity of diflubenzuron and tebufenozide is well characterized in most groups of animals, including mammals, birds, terrestrial invertebrates, fish, and aquatic invertebrates. In general, both of these compounds are much more toxic to some invertebrates, specifically arthropods, than to vertebrates or other groups of invertebrates.

This differential toxicity of these two compounds involves fundamentally different and well understood mechanisms of action, with tebufenozide being somewhat more selective than diflubenzuron. Toxicity of diflubenzuron to sensitive invertebrate species is based on the inhibition of chitin synthesis. Chitin is a polymer (repeating series of connected chemical

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subunits) of a glucose-based molecule and is a major component of the exoskeleton, outer body shell, of all arthropods. The inhibition of the formation of chitin disrupts the normal growth and development of insects and other arthropods. Both terrestrial and aquatic arthropods are affected, but there seems to be some substantial differences in sensitivity. The toxicity of tebufenozide to sensitive invertebrates is based on the mimicking of 20-hydroxyecdysone, an invertebrate hormone that controls molting. The effectiveness of tebufenozide in mimicking 20-hydroxyecdysone activity, however, appears to vary markedly among orders and species of invertebrates. In general, moths are sensitive to tebufenozide but other insects are much less sensitive.

The most sensitive effects in wildlife mammalian species and possibly other vertebrates exposed to diflubenzuron or tebufenozide are likely to be the same as those in experimental mammals (i.e., effects on the blood). The major difference between the hazard identification for diflubenzuron and tebufenozide concerns potential reproductive effects. As noted in the comparative human health risk assessment, tebufenozide may cause reproductive effects in mammals, while this effect has not been noted for diflubenzuron. Similarly, the reproductive effects of tebufenozide but not diflubenzuron are of concern for birds, although the data are somewhat inconsistent. The available studies on tebufenozide include a reproduction study investigating effects in mallard ducks and two reproduction studies investigating effects in bobwhite quail. In one of the quail studies, dietary concentrations of 300 and 1000 ppm caused reproductive effects. The effects were not observed in that study at 100 ppm; moreover, the effects were not observed in the more recent quail study or in the study on mallard ducks. A field study regarding the effects of tebufenozide on reproductive performance in birds noted trends that were not statistically significant but, nonetheless, suggestive of adverse reproductive effects in a warbler species. Thus, consistent with the interpretation by the U.S. EPA, reproductive effects in both mammals and birds are considered endpoints of concern for tebufenozide. For diflubenzuron, there is only one relatively old report of reproductive effects in birds and the effects reported have not been noted in other studies. Thus, also consistent with the approach taken by U.S. EPA, reproductive effects are not identified as an endpoint of concern for diflubenzuron.

Terrestrial invertebrates appear to be much more sensitive to diflubenzuron and tebufenozide than are vertebrates, and tebufenozide appears to affect a narrower group of invertebrates than does diflubenzuron. The terrestrial species most sensitive to diflubenzuron are arthropods, a large group of invertebrates, including insects, crustaceans, spiders, mites, and centipedes. In terrestrial organisms, the species most sensitive to diflubenzuron include lepidopteran and beetle larvae, grasshoppers, and other herbivorous insects. More tolerant species include bees, flies, parasitic wasps, adult beetles, and sucking insects. Tebufenozide is toxic to a much narrower range of terrestrial insects. In general, moths are sensitive to tebufenozide but other insects are much less sensitive.

Both diflubenzuron and tebufenozide are also more toxic to aquatic invertebrates than they are to fish. U.S. EPA has classified diflubenzuron as practically non-toxic to fish, with LC_{50} values that

range from 25 to 500 mg/L. Tebufenozide is somewhat more toxic to fish, with LC_{50} values that range from 2.2 to 6.5 mg/L for fish, categorized as moderately toxic using the U.S. EPA classification system. Invertebrates are affected at much lower concentrations and the relative potency of the two compounds is reversed, with diflubenzuron being substantially more toxic than tebufenozide to aquatic invertebrates. The NOEC values in invertebrates for diflubenzuron are as low as 0.3 µg/L in acute studies and 0.04 µg/L in longer-term studies. Tebufenozide is substantially less toxic to invertebrates, with NOEC values as low as 120 µg/L in acute studies and 3.5 µg/L in longer-term studies.

4.1.3.2. DDVP – Although DDVP is a general neurotoxin, the available data suggest that invertebrates are more sensitive than other organisms to DDVP. For example, the oral LD_{50} in honey bees is 0.29 mg/kg bee, and the topical LD_{50} is 0.65 mg/kg bee. Although DDVP is also toxic to birds, the oral LD_{50} value is about 10 mg/kg for the most sensitive species. Short-term repeat dose studies in mammals found that oral exposures to doses below about 0.5 mg/kg-day or inhalation exposures to 1–2 mg/m³ generally do not result in adverse effects. Thus, no effects are apparent in experimental mammals at doses that are clearly lethal to bees.

Aquatic animals are also sensitive to DDVP. As with terrestrial animals, invertebrates appear to be more sensitive than vertebrates. The lowest reported LC_{50} value in fish is approximately 0.2 mg/L. Some aquatic invertebrates are much more sensitive than fish to DDVP. For daphnids, the most sensitive group of invertebrate species, reported EC_{50} values range from 0.00007 to 0.00028 mg/L.

Most of the toxicity data on ecological receptors is limited to free DDVP, rather than a slow-release formulation like the Vaportape II product used in USDA programs to control the gypsy moth. Hence, the toxicity values reported for indicator species are likely to be conservative (i.e., suggest greater toxicity), as compared with Vaportape II. Although U.S. EPA assessed the ecological effects of DDVP, the exposures assessed are not specific to formulations in which DDVP is encapsulated in PVC resin. In general, aside from those organisms that enter the milk carton trap or those that remove the PVC strip form the trap, toxicity resulting from exposure of ecological receptors to DDVP in Vaportape II milk carton traps is not likely.

4.1.3.3. Disparlure – There is very little information regarding the toxicity of disparlure to nontarget wildlife species. As noted in the human health risk assessment, the U.S. EPA does not require extensive testing of insect pheromones. Thus, the only studies available are acute studies in bobwhite quail, mallard ducks, rainbow trout, bluegill sunfish, *Daphnia magna*, and Eastern oysters. No chronic exposure studies were identified.

Results of acute gavage and dietary exposure studies in mallard ducks and bobwhite quail show that disparlure has very low toxicity in these species, with no mortalities observed following exposure to up to 2510 mg/kg in bobwhite quail. Limited data are available regarding the toxicity disparlure to aquatic animals. Relative to mammals and birds, *Daphnia* appear to the

most sensitive species tested, with an LC_{50} value of 0.098 mg/L. In rainbow trout, 20% mortality was noted at a concentration of 100 mg/L.

4.2. EXPOSURE ASSESSMENT
4.2.1. Overview
Table 4-2 summarizes the exposure assessments on nontarget species for each of the agents covered in the risk assessment. Table 4-2 is similar to the corresponding table for the human health risk assessment (see Table 3-2) because the applications and uses for each control agent are identical. Since diflubenzuron, tebufenozide, LdNPV, and disparlure can be applied in broadcast applications, exposure potential is high and in many cases unavoidable, as is true for the human health risk assessment. When disparlure is used as an attractant in traps, exposures will be variable and primarily incidental. Exposures to the gypsy moth itself are also variable and depend on the extent of the gypsy moth population, which can range from low level infestation to outbreak conditions.

There are, however, notable differences between the human health exposure assessment and the ecological exposure assessment. Unlike Table 3-2, Table 4-2 does not provide measures of exposure for each agent, because the measures of exposure for ecological effects vary not only among the control agents but also among the target groups for each agent. For example, exposures to the gypsy moth are measured as egg masses/acre in the human health risk assessment, which is the same measure of exposure used for terrestrial vegetation, because it is the primary determinant in the dose-response assessment for plants. For all other species, however, effects from the gypsy moth are most likely to be secondary, which means the exposure assessment for these indirectly affected species is based on defoliation—i.e., the result of the dose-response assessment for terrestrial vegetation is used as the exposure assessment for most other groups of organisms.

Other differences in the exposure assessments for nontarget species are mostly superficial. For each of the chemical agents, the mass of the chemical is typically used as the measure of exposure. Depending on the group, the measure of exposure may be expressed as dose (mg agent/kg bw for most terrestrial species), concentration (mg agent/L of water for aquatic species), or simply as application rate (lb agent/acre). This last measure is used primarily when field studies are the basis for the dose-response assessment.

As in the human health risk assessment, different measures of exposure are used for each of the biological agents. For *B.t.k.*, most of the exposures are characterized simply as an application rate in units of BIU/acre. Nevertheless, colony forming units are used for some of the mammalian exposure scenarios. Also as in the human health risk assessment, no clear hazard potential is identified for LdNPV. The very few exposure scenarios that are quantified in the ecological risk assessment for LdNPV are based on the mass of the formulation, Gypchek.

The level of detail used in the exposure assessments for the different chemicals reflects differences in the use patterns and the nature of the available toxicity data. Full sets of exposure assessments in several groups of animals are developed for diflubenzuron and tebufenozide. As in the human health risk assessment, the exposure assessment for diflubenzuron is elaborated by

the consideration of 4-chloroaniline and the exposure assessment for tebufenozide is elaborated by the consideration of multiple applications.

Disparlure, which may also be applied in aerial broadcast applications, has a much more restricted set of exposure scenarios on far fewer groups of organisms. This difference is due completely to the sparse toxicity data available on this compound. In other words, while a very elaborate set of exposure scenarios could be prepared, these scenarios would serve little purpose because they could not be combined with a dose-response assessment to characterize risk. The exposure assessment for DDVP is also restricted due to the limited number of plausible exposures, given that DDVP is used only in milk carton traps and minimal exposures for nontarget species are anticipated under ordinary conditions.

4.2.2. Biological Agents

4.2.2.1. Gypsy Moth – As in the human health risk assessment, the exposure metameter is dictated by the data used to formulate the dose-response assessment—i.e., egg mass density is the exposure metameter for terrestrial invertebrates and plants because it is the measure on which the dose-response assessment is based. Egg mass densities ranging from 5 to 5000 egg masses/acre are used to estimate responses in terrestrial plants and invertebrates.

Most wildlife species are not affected directly by exposure to the gypsy moth but are more likely to experience indirect effects due to changes in habitat or other environmental conditions secondary to defoliation. Consequently, the exposure assessment for most wildlife species is almost identical to the dose-response assessment for terrestrial plants which is expressed as defoliation caused by gypsy moth larvae. For this exposure assessment, categories of defoliation are defined as normal background defoliation (<30% defoliation), moderate defoliation (30-60% defoliation), and high or severe defoliation (>60% defoliation).

4.2.2.2. B.t.k. – Based on the hazard identification, exposure assessments are presented for three groups: small mammals, terrestrial insects, and aquatic species. While a number of different exposure scenarios could be developed for terrestrial mammals, the only positive hazard identification for *B.t.k.* involves inhalation exposures. As in the human health risk assessment, inhalation exposures ranging from 100 to 5000 cfu/m^3 are used to assess potential risks of serious adverse effects in terrestrial vertebrates. These concentrations are applied to a 20 g mouse and correspond to inhaled doses of 0.00336-0.168 cfu/mouse. While there is no basis for asserting that any oral and/or dermal exposures are likely to cause adverse effects in terrestrial vertebrates, an extremely conservative exposure assessment is developed for combined oral (water and vegetation) and dermal (direct spray) exposures that yields an estimated maximum dose of about 184 mg/kg body weight. For terrestrial insects, the toxicity values used to assess the consequences of observing effects is given in units of BIU/ha. Consequently, the exposure assessment for this group is simply the range of application rates used in USDA programs —i.e., approximately 49-99 BIU/ha. For aquatic organisms, toxicity data are expressed in several different units such as mg formulation/L, IU/L, and cfu/L. Based on application rates used in USDA programs and conservative assumptions concerning the depth of water over which *B.t.k.*

might be sprayed, concentrations in water would be expected to be at or below 0.24 mg formulation/L. As discussed in the hazard identification, there is no basis for asserting that adverse effects in birds, plants, soil microorganisms, or soil invertebrates other than insects are of plausible concern. Consequently, explicit exposure assessments are not conducted for those groups.

4.2.2.3. LdNPV – Numerous wildlife species might be exposed to Gypchek or LdNPV as a result of ground and aerial applications of the Gypchek formulation. The need for any formal risk assessment is questionable, however, because neither Gypchek nor LdNPV appear to cause systemic adverse effects. Nonetheless, to provide some basis for comparing the potential risks of Gypchek with other agents used to control the gypsy moth, two extreme exposure assessments are developed: one for a terrestrial herbivore consuming contaminated vegetation and the other for aquatic organisms in a small pond directly sprayed with Gypchek at the highest application rate. For the terrestrial herbivore, the dose estimates range from 1.1 to 3.2 mg Gypchek /kg bw. For aquatic organisms, concentrations are expressed in units of PIB/L because this unit is used in the corresponding toxicity studies. For a small pond directly sprayed with Gypchek at the highest application rate, the estimated initial concentration is 2.5×10^5 PIB/L. Several less extreme exposure assessments could be developed but they would not alter the risk assessment given that the extreme exposure assessments are substantially below any level of concern.

4.2.3. Chemical Agents
4.2.3.1. Diflubenzuron and Tebufenozide – As in the human health risk assessment, the exposure assessments for diflubenzuron and tebufenozide are similar. The same set of exposure scenarios are used with the same set of potential target species. The difference in their application rates dominates the quantitative difference in projected exposure to these two chemicals: a single application rate of 0.0625 lb/acre for diflubenzuron and one or two applications at 0.12 lb/acre for tebufenozide. As a result of the higher application rate for tebufenozide, all exposures are higher for tebufenozide than for diflubenzuron. Also as in the human health risk assessment, the exposure assessments for diflubenzuron are elaborated to include 4-chloroaniline as an environmental metabolite of diflubenzuron.

Not withstanding the quantitative differences in the application rates, the patterns of exposure for terrestrial species for diflubenzuron and tebufenozide are similar except for the maximum acute exposure. For diflubenzuron, this exposure is associated with direct spray of a small mammal and could reach 10 mg/kg. For tebufenozide, the maximum acute exposure is associated with a fish-eating bird and could be as high as 85 mg/kg. For other acute and longer-term exposures, the consumption of contaminated vegetation results in higher levels of exposure to both compounds than does the consumption of contaminated water. Estimates of longer-term daily doses for a small mammal consuming contaminated vegetation at the application site range up to 0.005 mg/kg for diflubenzuron and 0.08 mg/kg/day for tebufenozide. The consumption of contaminated water by a small mammal results in estimated doses of up to 0.00001 mg/kg/day for diflubenzuron and 0.0002 mg/kg/day for tebufenozide. Exposures of terrestrial organisms to 4-chloroaniline as a degradation product of diflubenzuron tend to be much lower than the doses

for diflubenzuron. The highest acute exposure to 4-chloroaniline is about 0.2 mg/kg, the approximate dose for the consumption of contaminated water by a small mammal and the consumption of contaminated fish by a predatory bird. The highest longer term exposure to 4-chloroaniline is 0.0002 mg/kg/day, the dose associated with the consumption of contaminated vegetation by a large bird.

As dicussed in Section 4.3, the toxicity data on terrestrial invertebrates are much more extensive for diflubenzuron than tebufenozide, which is directly related to differences in the numbers of field studies available on diflubenzuron (many), compared with tebufenozide (very few). The difference reflects the long-time, extensive use of diflubenzuron, compared with tebufenozide, which is a more recently introduced insecticide. For both chemicals, exposure of terrestrial invertebrates is generally expressed as an application rate from a field study, and no formal exposure assessment is given.

Exposures of aquatic organisms to diflubenzuron or tebufenozide are based essentially on the same information used to assess the exposures of terrestrial species from contaminated water. At the maximum application rates, the upper range of the expected peak concentration in surface water is estimated at 16 µg/L for diflubenzuron and 40 µg/L for tebufenozide.

4.2.3.2. Disparlure – Given the apparent low acute toxicity of disparlure and the lack of any chronic toxicity data, an exposure assessment for terrestrial species would not add to the assessment of risk. Acute exposure studies in *Daphnia* and rainbow trout show that aquatic animals appear more sensitive than terrestrial animals to disparlure. Therefore, an exposure assessment for aquatic species is made based on aerial spray of a pond at an application rate of 29.1 g a.i./acre, with an estimated concentration in pond water of 0.0072 mg a.i./L.

4.2.3.3. DDVP – As in the human health risk assessment, exposure of terrestrial mammals to DDVP from the VaporTape strips used in milk carton traps is likely to be negligible under most circumstances. Nonetheless, it is conceivable that some mammals such as racoons or bears could easily access and tamper with the milk carton trap. Depending on the proportion of the DDVP strip that is consumed, doses (as DDVP in the PVC strip) are estimated to range from 10.5 mg/kg (10% of strip) to 105 mg/kg (100% of strip) and the central estimate is taken as 31.6 mg/kg (30% of strip). In addition, contamination of water with a pest strip is plausible, although probably rare, and is considered in a manner similar to the corresponding scenario in the human health risk assessment (see Section 3.2.3.4). This scenario is based on the consumption of contaminated water by a small mammal, and the dose to the animal is estimated at about 0.00003 mg/kg with a range from 0.000009 to 0.00009 mg/kg. Other exposure scenarios for terrestrial vertebrates, while possible, seem far less plausible and are not considered quantitatively. No quantitative exposure assessments for terrestrial invertebrates are developed because the milk carton trap will attract only male gypsy moths. Nontarget insects that incidentally enter the trap are likely to be killed by exposure to the DDVP vapor. Exposures to aquatic species are based on the same water concentrations used for terrestrial species: 0.000177 mg/L with a range from 0.000059 to 0.00059 mg/L.

4.3. DOSE-RESPONSE ASSESSMENT
4.3.1. Overview
An overview of the dose-response assessment for groups of nontarget species is presented in Table 4-3. The information in this table categorizes the data descriptively rather than in terms of data quality. The categories reflect whether the data are sufficient to quantify risk or quantitatively characterize differences in sensitivity among several species in the designated group (●), whether the dose-response assessment is based on both an effect and no-effect level (■), whether the dose-response assessment is based only on a no-effect level (□), or whether the assessment is based only on an effect level (○). These categories are reasonable measures of data quality for all of the agents covered in this risk assessment except LdNPV.

All of the risk values for LdNPV are based on no- effect concentrations or doses. In general, confidence in any dose-response relationship is enhanced if a clear dose-response relationship can be demonstrated and both effect and no-effect exposures have been identified. In the case of LdNPV, however, there is simply no indication that LdNPV or the Gypchek formulation will cause toxicity in any nontarget species at any dose level. While additional studies could be conducted at higher doses and while these studies would enhance confidence in the risk assessment, the NOAEL and NOEC values that have been identified are far above any plausible exposures. Thus, while based on limited data in terms of the dose-effect characterization, the dose-response assessment for LdNPV is adequate for risk characterization.

For most of the other agents, the dose-response assessments are reasonably good for the species of greatest concern. As noted in Table 4-3, dose-response assessments for DDVP are derived only for mammals, fish, and aquatic invertebrates. As discussed in the exposure assessment, this limited approach is taken with DDVP because of the limited use of DDVP in programs to control the gypsy moth. The DDVP is contained in a PVC strip that is placed in a milk carton trap that includes disparlure as an attractant for the gypsy moth. This type of use limits potential exposure for most nontarget species. A formal dose-response assessment is not conducted for terrestrial invertebrates. This is not due to any lack of data. The toxicity of DDVP to insects and many other invertebrates is very well characterized. DDVP is such a potent insecticide that no formal dose-response assessment is needed. Insects and many other species that enter the trap are likely to be killed by exposure to DDVP.

Disparlure is the other agent for which a full set of dose-response assessments are not conducted. As discussed in the hazard identification, this is due to the limited amount of data regarding the toxicity of disparlure to nontarget species.

Relatively full dose-response assessments on groups of greatest concern are given for the gypsy moth, *B.t.k.*, diflubenzuron and its 4-chloroaniline metabolite, and tebufenozide. For the gypsy moth, the effect of primary concern is damage to vegetation. While data are available on both lethality in trees as well as defoliation, defoliation is used as the sublethal effect of primary concern. A dose-response assessment is also given for nontarget lepidopterans. While effect and

no-effect levels can be identified, the significance of this effect is questionable. In terms of direct effects, terrestrial vegetation is the primary target of concern.

Lepidoptera are the primary nontarget group of concern for *B.t.k.* exposure. A relatively rich set of studies is available regarding the sensitivities of nontarget *Lepidoptera* and some other insects. The sensitivities of the nontarget insects can be quantified reasonably well from exposures that encompass the application rates used in USDA programs to control the gypsy moth. Sensitive nontarget *Lepidoptera* include larvae of the endangered Karner blue butterfly as well as several other types of moths.

Similar types of information are available on diflubenzuron and tebufenozide, and dose-response assessments can be made for the species of primary concern. For both chemicals, this includes nontarget *Lepidoptera* and aquatic invertebrates. Other terrestrial arthropods are also considered for diflubenzuron. In addition, because of the standard tests required by U.S. EPA for the registration of most pesticides, adequate toxicity data are available on mammals, birds, and fish. The toxicity data base for diflubenzuron is somewhat more extensive and sensitivities in nontarget organisms are somewhat better defined in both laboratory and field studies than is the case with tebufenozide.

4.3.2. Biological Agents

4.3.2.1. Gypsy Moth – As in the human health risk assessment for the gypsy moth, the dose measure for the gypsy moth is egg masses/acre. Quantitative dose-response assessments can be made for both terrestrial plants and sensitive species of *Lepidoptera*. The dose-response assessments for terrestrial plants are based on a relatively simple quantitative model for the relationship of egg mass density and vegetation type to defoliation. Three broad categories of vegetation (sensitive, intermediate, and tolerant) are used to characterize the susceptibility of forest stands to gypsy moth induced defoliation. Estimated LOAEL values based on 30% defoliation, which is considered the lower range of moderate defoliation, are approximately 125 egg masses/acre for sensitive stands, 1000 egg masses/acre for intermediate stands, and 7000 egg masses/acre for tolerant stands. The corresponding NOAEL values, defined as 10% defoliation, are estimated as 12, 20, and 125 egg masses/acre for sensitive, intermediate, and tolerant forest stands.

The effects of gypsy moth exposure on sensitive terrestrial invertebrates, including some species of *Lepidoptera*, are less well documented and less well characterized, compared with the effects on terrestrial plants. Nonetheless, available studies indicate that the NOAEL for adverse effects in certain other species of *Lepidoptera* are lower than the NOAEL for sensitive forest stands–– i.e., about 6-72 egg masses/acre for some *Lepidoptera*. No quantitative dose-response assessment is presented for other groups of organisms—e.g., mammals, birds, and soil or aquatic organisms. The impact of gypsy moth exposure on these species is most likely to result in indirect effects secondary to defoliation. This is discussed further in the risk characterization.

4.3.2.2. B.t.k. – As summarized in Table 4-3, exposure assessments are presented for four groups: mammals, terrestrial insects, fish, and invertebrates. While a number of different exposure scenarios could be developed for terrestrial mammals, the only positive hazard identification for *B.t.k.* involves inhalation exposures. As in the human health risk assessment, inhalation exposures of 100-5000 cfu/m^3 are used to assess potential risks of serious adverse effects in terrestrial vertebrates. These concentrations are applied to a 20 g mouse and correspond to inhaled doses of 0.00336-0.168 cfu/mouse. While there is no basis for asserting that any oral and/or dermal exposures are likely to cause adverse effects in terrestrial vertebrates, an extremely conservative exposure assessment is developed for combined oral (water and vegetation) and dermal (direct spray) exposures that yields an estimated maximum dose of approximately 184 mg/kg body weight.

For terrestrial insects, the toxicity values used to assess the consequences of observing effects is given in units of BIU/ha over a range of applications similar to those used in gypsy moth control programs. The magnitude of response to *B.t.k.* in sensitive nontarget species appears similar to that of the gypsy moth. Tolerant species appear to be about 30-fold less sensitive than the gypsy moth to B.t.k.. The designations of sensitive and tolerant species are not intended to imply absolute ranges on tolerance among all possible insects. Instead, the dose-response assessments for this group simply indicate that some nontarget species, such as the Karner blue butterfly and cinnabar moth, appear to be as sensitive to *B.t.k.* as target species such as the gypsy moth and cabbage looper. The range of sensitivities among various insect species appears to follow a continuum, and it is possible that some species may be more or less sensitive to *B.t.k.* than those insects on which toxicity data are available.

For aquatic organisms, toxicity data are expressed in several different units such as mg formulation/L, IU/L, and cfu/L. Based on application rates used in USDA programs and conservative assumptions concerning the depth of water over which *B.t.k.* might be sprayed, concentrations in water would be expected to be at or below 0.24 mg formulation/L. Toxicity values for fish are 1.4 mg formulation/L (an LOEC for sensitive species) and 1000 mg formulation/L (an NOEC for tolerant species). For aquatic invertebrates, the NOEC values for sensitive and tolerant species are 0.45 and 36 mg/L, respectively.

4.3.2.3. LdNPV – Because no hazards can be identified for any species, a quantitative dose-response assessment is not required. Consequently, no dose-response assessments were proposed by U.S. EPA and none were used in the previous gypsy moth risk assessment for Gypchek. In order to provide a quantitative comparison of the risks of using Gypchek relative to the other agents, dose-response assessments are made for both terrestrial mammals and aquatic species. For terrestrial mammals, the NOAEL of 2600 mg/kg bw is used. This is the same NOAEL that serves as the basis for the surrogate acute RfD for LdNPV in the human health risk assessment for this agent. For aquatic species, only NOEC values are available, and the highest NOEC of 8×10^9 PIB/L is used to characterize risk.

4.3.3. Chemical Agents

4.3.3.1. Diflubenzuron and Tebufenozide – As summarized in Table 4-3, the dose-response assessments for diflubenzuron and tebufenozide are far more complete, in terms of the number of groups encompassed, than are the corresponding assessments for other agents considered in this risk assessment. This difference reflects both the nature of the available data and an assessment of the need to characterize risk quantitatively. Despite their specific modes of action in target species, diflubenzuron and tebufenozide induce toxicological responses in many different groups of animals. Furthermore, both chemicals are used in broadcast aerial applications, making exposure to many different groups of organisms likely.

Both diflubenzuron and tebufenozide are relatively non-toxic to mammals and birds. As noted in the human health risk assessment, the acute and chronic toxicities of these two chemicals in mammals appear to be virtually identical in terms of NOAELs. This is also true for birds. The toxicity values used in the ecological risk assessment for mammals are identical to those used in the human health risk assessments: an acute NOAEL of 1118 mg/kg and a chronic NOAEL of 2 mg/kg/day for diflubenzuron and an acute NOAEL of 1000 mg/kg and a chronic NOAEL of 1.8 mg/kg/day for tebufenozide. The differences between the values for the chemicals are clearly insubstantial. For birds, the acute NOAEL for diflubenzuron is taken as 2500 mg/kg and the longer-term NOAEL is taken as 110 mg/kg/day. For tebufenozide, the values are again very similar: an acute NOAEL of 2150 mg/kg and a longer-term NOAEL of 15 mg/kg/day. For both chemicals, the longer-term NOAEL is taken from standard assays on reproduction.

In terms of potential effects on terrestrial invertebrates, the data set for diflubenzuron is much richer than the data set for tebufenozide. Many laboratory toxicity studies and field studies have been conducted on diflubenzuron. Field studies are used in the dose-response assessment of diflubenzuron because the standard toxicity studies are extremely diverse and many are not directly applicable to a risk assessment. Despite the difficulty and uncertainty in interpreting some of the field studies, the relatively large number of field studies on diflubenzuron appears to present a reasonably coherent pattern that is at least qualitatively consistent with the available toxicity data and probably a more realistic basis on which to assess risk to nontarget species. The most sensitive species appear to be grasshoppers which may be adversely affected at an application rate of about 0.02 lb/acre [22 g/ha]. Somewhat high application rates—in the range of 0.027-0.031 lb/acre [30 to 35 g/ha]—will adversely affect macrolepidoptera and some beneficial parasitic wasps. At the maximum application rate considered in this risk assessment— 0.062 lb/acre [70 g/ha]—some additional herbivorous insects are likely to be affected. No adverse effects in several other groups of insects are expected at this or much higher application rates. Honeybees are among the most tolerant species and are not likely to be adversely affected at application rates of up to 0.35 lb/acre [400 g/ha]. Invertebrates that do not synthesize chitin are also relatively tolerant to diflubenzuron.

Although there are fewer and generally less detailed field studies on tebufenozide, compared with diflubenzuron, it appears to be less toxic to nontarget species (e.g., lacewing). In general, the field studies indicate that tolerant insect species are not affected by tebufenozide at application

rates up to 0.24 lb/acre. The true NOEC may be higher – i.e., an LOEC has not been identified for tolerant species of terrestrial insects. Conversely, application rates as low as 0.03 lb/acre have been shown to have adverse effects on sensitive nontarget insects, primarily *Lepidoptera*. A NOEC for sensitive species was not identified.

For both diflubenzuron and tebufenozide, the toxicity values for aquatic species follow a pattern similar to that for terrestrial species: arthropods appear to be much more sensitive than fish or non-arthropod invertebrates. Both compounds are about equally toxic to fish with virtually identical chronic NOEC values: 0.05 mg/L for diflubenzuron and 0.048 mg/L for tebufenozide.

There are major and substantial differences regarding the toxicity of diflubenzuron and tebufenozide to aquatic invertebrates. Diflubenzuron is much more toxic. In acute toxicity studies, the NOEC for the most sensitive species is 0.0003 mg/L diflubenzuron, which is 400 times less than the corresponding NOEC of 0.12 mg/L for tebufenozide. Chronic toxicity studies indicate a similar pattern. The NOEC for the most sensitive species is 0.00004 mg/L for diflubenzuron and 0.0035 mg/L for tebufenozide. The difference is a factor of about 90 [0.0035 mg/L / 0.00004 mg/L]. Even though the number of available NOEC values is greater for diflubenzuron (seven acute and seven chronic), compared with tebufenozide (three acute and two chronic), and variability can be expected to increase as the number of species tested increases, it is unlikely that the apparent differences in toxicity are artifacts of sample size. For example, based on acute and chronic NOEC values in *Daphnia*, which are available for both compounds, diflubenzuron is more toxic than tebufenozide by a factor of about 2700 in acute studies and a factor of 725 in chronic studies. The toxicity to aquatic invertebrates is one of the few areas in which diflubenzuron and tebufenozide differ remarkably, and this difference has an impact on the risk characterization (Section 4.4).

4.3.3.2. Disparlure – The limited amount of toxicity data on disparlure precludes making a standard dose-response assessment for terrestrial species. Disparlure is identical or similar to pheromones produced by other species of moths and is able to attract male nun moths. Since, however, there are no quantitative data available regarding the efficacy of disparlure in nontarget moths, a dose-response assessment for this effect in a nontarget species of moths cannot be made. For aquatic species, NOEC values and limited data on effect levels are available from acute exposure studies in rainbow trout and *Daphnia*. No LC_{50} values are available in fish. The dose-response assessment is limited to NOEC values of 10 mg/L in trout and 300 mg/L in bluegills. The only information on toxic effects in fish consists of a report of 20% mortality in trout after acute exposure to disparlure at 100 mg/L. Thus, disparlure does not appear to be highly toxic to fish. *Daphnia magna* are much more sensitive with a 48-hour LC_{50} of 0.098 mg/L and an NOEC for mortality of 0.017 mg/L. Based on the LC_{50} value, disparlure is classified as highly toxic to aquatic invertebrates.

4.3.3.3. DDVP – Given the limited nature of the use of DDVP in programs to control the gypsy moth and consequent limited number of exposure assessments, the dose-response assessment for DDVP is relatively simple. For terrestrial mammals, a value of 240 mg/kg from a study using

DDVP in a PVC formulation is used for direct exposure to the DDVP-PVC strip—i.e., a raccoon tampering with a milk carton trap and consuming all or part of the DDVP strip. At the dose of 240 mg/kg, no mortality or frank signs of AChE inhibition were observed. For the contaminated water scenario, the NOAEL of 0.5 mg/kg from a study involving exposure to free or unformulated DDVP is used. This NOAEL is from the study that forms the basis for the acute RfD used in the human health risk assessment. Although DDVP is classified as highly toxic to fish, the estimated levels of acute exposure for fish are far below the 30-day NOEC of 0.03 mg/L. Thus, this value is used for all fish and no attempt is made to consider differences in sensitivity among fish. A somewhat different approach is taken with aquatic invertebrates, some of which are more sensitive than fish to DDVP by a factor of more than 2500. Risks to sensitive species of aquatic invertebrates —i.e., daphnids and other small arthropods—are characterized based on the lowest reported LC_{50} value, 0.00007 mg/L from a 48-hour bioassay in *Daphnia pulex*. Some other groups of aquatic invertebrates, such as snails, appear to be much less sensitive than small arthropods. Risks to such tolerant species are based on a LC_{50} value of 21 mg/L in a freshwater snail.

4.4. RISK CHARACTERIZATION
4.4.1. Overview
The comparative risk characterization for the ecological risk assessment is expressed similarly to that in the human health risk assessment. Numerically, the risk characterizations are given as hazard quotients (HQs), the level of exposure divided by some measure of effect, typically an NOAEL or NOEC. As in the human health risk assessment, the comparative risk characterization for ecological effects typically categorizes concern with the agents as marked (HQ>10), marginal (HQs between about 0.1 and 10), and minimal (HQ<0.1). One exception is made for *B.t.k.*, which is classified as an agent of marked concern although the highest HQ is 9.4.

An overview of the comparative risk characterization is summarized in Table 4-4 for terrestrial species and Table 4-5 for aquatic species. The risk characterizations are illustrated in Figure 4-1 (terrestrial) and Figure 4-2 (aquatic). As in the human health risk assessment, the HQs for each agent are presented as a range. The upper end of the range is typically the highest hazard quotient associated with a plausible exposure scenario. The lower end of the range is not necessarily the lowest HQ calculated in each of the risk assessments. For some agents, the lower range is taken from sets of exposure scenarios that provide similar HQs for exposures that may be regarded as typical. For these agents, the lowest HQs reported in the individual risk assessments are close to zero. In some cases, the numerical expressions of risk do not adequately convey the potential for hazard. These cases are noted in Figures 4-1 and 4-2 with comments.

Ecological risk assessments involve, at least implicitly, considerations of thousands of different species and the relationships among these species and their habitats. Invariably, however, data are available on only a small subset of these species and field studies provide only limited insight into the complex interrelationships and secondary effects among species. Thus, as in the human health risk assessments, ecological risk assessments cannot offer a guarantee of safety. They can and do offer a means to identify whether or not there is a basis for asserting that adverse effects are plausible and what the nature of these effects might be.

Within these limitations, only LdNPV clearly qualifies as an agent of minimal concern. While there are limitations in the available studies on LdNPV, there is simply no basis for asserting that LdNPV will adversely affect any species except the gypsy moth.

Agents of marked concern include the gypsy moth, *B.t.k.*, and diflubenzuron. The types of concern with each of these agents, however, are quite different. For both the gypsy moth and *B.t.k.*, the concerns are narrow. The gypsy moth clearly will damage some terrestrial vegetation. *B.t.k.* is likely to affect sensitive *Lepidoptera*. Concern with the use of diflubenzuron is broader and includes effects on both terrestrial and aquatic invertebrates.

The designation of the gypsy moth as an agent of marked concern is obvious. The effects of gypsy moth larvae on forests are extremely well documented and well understood. In sensitive forest stands—i.e., stands in which oak, birch, and other favored species predominate —gypsy moth larvae can cause substantial defoliation and tree mortality. While some other lepidopteran

species also may be directly affected by exposure to the gypsy moth, most of the other effects caused by the gypsy moth will be secondary. Reductions in populations of squirrels, mice, and other mammals which may be sensitive to changes in the availability of acorns are likely and have been well documented. Substantial secondary adverse effects on other groups of animals— i.e., birds, reptiles, and aquatic species—cannot be ruled out but have not been convincingly or consistently demonstrated.

Diflubenzuron is also clearly an agent of marked concern. Exposures to diflubenzuron at application rates used in gypsy moth control programs will adversely affect both terrestrial and aquatic invertebrates that rely on chitin for their exoskeleton. This has been demonstrated in controlled toxicity studies as well as multiple field studies.

The designation of *B.t.k.* as an agent of marked concern is somewhat judgmental. As noted in Table 4-4, the highest hazard quotient is 9.4. Based on this HQ and the classification scheme used generally, *B.t.k.* would be classified as an agent of marginal concern. However, recent studies convincingly demonstrate that adverse effects in nontarget *Lepidoptera* will occur in the applications of *B.t.k.* used to control the gypsy moth. Concern is heightened because some of the *Lepidoptera* that may be adversely affected include at least one endangered species.

Tebufenozide, DDVP, and disparlure are all classified as agents of marginal concern. For tebufenozide, the numerical expressions of risk may be less relevant than a more qualitative assessment. The highest HQ is 4 and is associated with the consumption of contaminated vegetation by a large mammal after two applications of the compound at the highest labeled application rate. While this exposure would be considered undesirable, it is not clear that any frank signs of toxicity would be seen. Risks to nontarget *Lepidoptera* may be of greater concern but the available data are insufficient to quantify potential risk. Risks to other invertebrates, both terrestrial and aquatic, appear to be insubstantial. DDVP is of marginal concern in that highly localized effects may be expected: nontarget insects entering a milk carton trap or some aquatic invertebrates affected by the accidental contamination of a small body of water with a pest strip. In both cases, the effects would be relatively minor, in terms of the number of organisms affected. Marginal concern for disparlure is associated with the relatively high toxicity of this agent to *Daphnia* and is reenforced by the very scant data on the toxicity of an agent that may be applied to large areas in broadcast applications.

4.4.2. Agents of Marked Concern
4.4.2.1. Gypsy Moth – The best documented and most obvious effect of the gypsy moth will be on terrestrial vegetation, particularly forest stands in which sensitive species of trees predominate. In some respects, the risk characterization for terrestrial vegetation is essentially a restatement of the hazard identification. In other words, the effects of gypsy moth larvae on forests is extremely well documented and relatively well understood. In sensitive forest stands—i.e., stands in which oak, birch, and other favored species predominate —gypsy moth larvae can cause substantial defoliation. In forest stands in which tree species that are not favored by gypsy moth larvae predominate —e.g., hemlock, various types of pine, black locust

and white ash—even relatively high exposures, as measured by egg mass density, may not result in substantial defoliation. The risk assessment for direct effects on forests should be at least qualitatively influenced by the current range of the gypsy moth, which has not yet extended to some forests in the southeast that may be among the most sensitive to gypsy moth exposure. Thus, unless measures to contain the gypsy moth are successful, the southeastern oak forests may suffer serious damage in future infestations.

Some other lepidopteran species also may be directly affected by exposure to the gypsy moth. Most studies, however, suggest that substantial adverse effects in terrestrial insects are unlikely and effects in some insect species, including some other *Lepidoptera*, may be beneficial.

Because the gypsy moth may substantially damage some forests in severe infestations or outbreaks, secondary effects in other species of wildlife are plausible. Reductions in populations of squirrels, mice, and other mammals which may be sensitive to changes in the availability of acorns are likely. Substantial adverse effects on other groups of animals—i.e., birds, reptiles, and aquatic species—cannot be ruled out but have not been convincingly demonstrated.

4.4.2.2. *B.t.k.* – Terrestrial insects are the only organisms likely to be adversely affected by exposure to *B.t.k.* or its formulations. Separate dose-response curves can be generated for both sensitive and tolerant terrestrial insects. At the application rates used to control gypsy moth populations, mortality rates among sensitive terrestrial insects are likely to range from approximately 80% to 94% or more. All sensitive terrestrial insects are *Lepidoptera* and include some species of butterfly, like the endangered Karner blue and some swallowtail butterflies and promethea moths.

The effects in sensitive species have been convincingly demonstrated in the study by Herms et al. (1997). In this study, the toxicity of Foray 48B was assayed in larvae of both the gypsy moth and the Karner blue butterfly, an endangered species of butterfly indigenous to the northern United States (Minnesota to New Hampshire). Bioassays in both species involved applications of Foray 48B to vegetation (wild lupine leaves for the Karner blue and white oak leaves for the gypsy moth) at treatment levels equivalent to either 30-37 BIU/ha per ha (low dose) or 90 BIU/ha (high dose). A negative control consisted of untreated vegetation. The insect larvae (either 1st or 2nd instar for the Karner blue and 2nd instar for the gypsy moth) were placed on the vegetation 7 to 8 hours after treatment and allowed to feed for 7 days. Survival rates for Karner blue larvae were: 100% for controls, 27% at the 30-37 BIU/ha treatment rate, and 14% at the 90 BIU/ha treatment rate. Survival rates for gypsy moth larvae were: 80% for controls; 33% for low-dose treatment, and 5% for high-dose treatment. Based on a statistical analyses of these data, the gypsy moth and Karner blue appear to be equally sensitive to *B.t.k.* This study is supplemented by the series of bioassays conducted by Peacock et al. (1998) which suggest that various other lepidopteran species may be as sensitive as the gypsy moth to *B.t.k.*.

For some *Lepidoptera*, sensitivity to *B.t.k.* is highly dependent on developmental stage. This is particularly evident for the cinnabar moth, where late instar larvae are very sensitive to *B.t.k.* and

early instar larvae are very tolerant to *B.t.k.* Given the mode of action of *B.t.k.*—i.e., it must be ingested to be highly toxic to the organism— effects on even the most sensitive species will occur only if exposure coincides with a sensitive larval stage of development. In tolerant species, including non-lepidopteran insects and certain larval stages of some *Lepidoptera*, the anticipated mortality rates are much lower (on the order of less than 1% to about 4%).

The risk characterization for terrestrial mammals is unambiguous: under foreseeable conditions of exposure, adverse effects are unlikely to be observed. Similarly, based on a very conservative exposure assessment for aquatic species, effects in fish and aquatic invertebrates appear to be unlikely. As discussed in the hazard identification, effects in birds, plants, soil microorganisms, or soil invertebrates other than insects are not of plausible concern. Thus, quantitative risk characterizations for these groups are not conducted. For oil-based formulations of *B.t.k.* (or any other pesticide), effects in some soil invertebrates are plausible.

4.4.2.3. Diflubenzuron – While the data base supporting the risk assessment of diflubenzuron is large and somewhat complex, the risk characterization is relatively simple and unequivocal. Diflubenzuron is an effective and general insecticide. Application rates used to control the gypsy moth are likely to have effects on some nontarget terrestrial insects. Species at greatest risk include grasshoppers, various macrolepidoptera (including the gypsy moth), other herbivorous insects, and some beneficial predators to the gypsy moth. These species are at risk because of the mode of action of diflubenzuron (i.e., inhibition of chitin) and the behavior of the sensitive insects (the consumption of contaminated vegetation or predation on the gypsy moth). Some aquatic invertebrates may also be at risk but the risks appear to be less than risks to terrestrial insects. The risk characterization for aquatic invertebrates is highly dependant on site-specific conditions. If diflubenzuron is applied when drift or direct deposition in water is not controlled well or in areas where soil losses from runoff and sediment to water are likely to occur, certain aquatic invertebrates are at risk of acute adverse effects, and exposure could cause longer-term effects on more sensitive species.

Direct effects of diflubenzuron on other groups of organisms—that is, mammals, birds, amphibians, fish, terrestrial and aquatic plants, microorganisms, and non-arthropod invertebrates—do not appear to be plausible. Nontarget species that consume the gypsy moth or other invertebrates adversely affected by diflubenzuron may be at risk of secondary effects (for example, a change in the availability of insect prey). There is no indication that 4-chloroaniline formed from the degradation of diflubenzuron will have an adverse effect on any terrestrial or aquatic species.

4.4.3. Agents of Marginal Concern
4.4.3.1. Tebufenozide – The use of tebufenozide to control the gypsy moth may result in adverse effects in nontarget *Lepidoptera* but these effects have not been well characterized or clearly demonstrated. There is little indication that other species will be impacted under normal conditions of use even at the highest application rate. Tebufenozide is an insecticide that is effective in controlling populations of lepidopteran pests. No data, however, are available on

toxicity to nontarget *Lepidoptera*. For the risk assessment of this compound, the assumption is made that nontarget *Lepidoptera* may be as sensitive as target *Lepidoptera* to tebufenozide. Thus, adverse effects in nontarget *Lepidoptera* would be expected after applications that are effective for the control of lepidopteran pest species.

There is no indication that short-term exposures to tebufenozide will cause direct adverse effects in any terrestrial vertebrates or non-lepidopteran invertebrates even at the upper range of plausible exposures or as a result of accidental exposures. Similarly, direct adverse effects from longer-term exposures in birds and mammals appear to be unlikely under most conditions. Effects on birds due to a decrease in available prey—i.e., terrestrial invertebrates—are considered plausible. In extreme cases, exposure levels in some large mammals might exceed the NOEC, but would remain below levels associated with frank signs of toxicity. This point is reflected in the HQ of 4 for a large mammal consuming contaminated vegetation after two applications of tebufenozide at the highest labeled rate. Under normal conditions of use, tebufenozide is not likely to cause adverse effects in aquatic species; however, in the case of a large accidental spill into a relatively small body of water, adverse effects might be expected in aquatic vertebrates, invertebrates, and plants.

4.4.3.2. DDVP – As in the human health risk assessment of DDVP, typical exposures and consequent risks to nontarget species should be negligible. The containment of the DDVP within a slow-release PVC strip combined with the target specific nature of pheromone baited traps should reduce the risks of inadvertent effects on nontarget species. Other insects and arthropods that may inadvertently enter the trap will probably be killed by DDVP vapor. While such inadvertent contact may occur, it is not likely to have a substantial impact on the number of nontarget insects or arthropods.

Because of the limited use of DDVP, a relatively small number of exposure scenarios —all of which might be considered accidental or incidental—are developed. For terrestrial mammals, contact with the pest strip could occur by an animal directly tampering with a trap or by an animal consuming water accidentally contaminated with a DDVP strip. Adverse effects would not be expected in either case. In the case of accidental contamination of a small body of water with a DDVP strip, concentrations of DDVP in the water would be below the level of concern for fish by factors ranging from about 50 to 500. Some aquatic invertebrates, however, might be affected. For the most sensitive species of aquatic invertebrates —i.e., small aquatic arthropods like daphnids—exposure levels could substantially exceed laboratory LC_{50} values by factors of up to about 8. Exposures to tolerant aquatic invertebrates—like snails—would be below a level of concern by a substantial margin—i.e., factors ranging from about 30,000 to 300,000.

The exposure assessments that serve as the bases for these risk characterizations are highly dependent on specific conditions—i.e., how much DDVP was in the strip at the time that the contamination occurred and the size of the body of water that was contaminated. Because the hydrolysis of DDVP in water is rapid, the estimates of adverse effects in some aquatic

invertebrates would probably apply only to a very limited area near the pest strip rather than to the larger area of the body of water that is contaminated.

4.4.3.3. Disparlure – There is little data available on terrestrial and aquatic animals to allow for a quantitative characterization of risk. The lack of chronic toxicity data in any species adds uncertainty to any risk characterization. Thus, for both terrestrial and aquatic species, the potential for the development of toxicity from long-term exposure to disparlure cannot be ruled out. Concern with the lack of toxicity data on disparlure is exacerbated by the fact that this compound may be applied to large areas in broadcast applications.

Nonetheless, based on the available data, clear hazards to nontarget species have not been identified. Disparlure may disrupt mating in some moths other than the gypsy moth. The two species that are known to be affected, however, are both forest pests like the gypsy moth and only one of these other species is native to North America. For aquatic species, hazard quotients for both rainbow trout and *Daphnia* are below one, although the hazard quotient of 0.4 for *Daphnia* approaches one. Thus, while 0.4 is below the level of concern of one, there is uncertainty in the risk characterization because of the limited acute toxicity data, the lack of chronic toxicity data, and the high likelihood that many species will be exposed to this compound.

4.4.4. Agent of Minimal Concern: Gypchek

Unlike all of the other agents considered in this risk assessment, there is no basis for asserting that the use of Gypchek to control or eradicate gypsy moth populations is likely to cause any adverse effects in any species other than the gypsy moth. While no pesticide is tested in all species under all exposure conditions, the data base on LdNPV and related viruses is reasonably complete and LdNPV has been tested adequately for pathogenicity in a relatively large number of species, particularly terrestrial invertebrates. LdNPV appears to be pathogenic and toxic to the gypsy moth and only to the gypsy moth.

For Gypchek, quantitative expressions of risk are in some respects more difficult because clear LOEC values are not defined—i.e., if an agent is not shown to cause an effect, the threshold for effects is not a meaningful concept. Nonetheless, general but very conservative exposure assessments demonstrate that plausible upper ranges of exposures are clearly below any level of concern by a factor of 1000 for terrestrial species and 30,000 for aquatic species.

5. REFERENCES

ATSDR (Agency for Toxic Substances and Disease Registry). 2004. Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures. Available from U.S. Department of Health and Human Services, Public Health Service, ATSDR, Division of Toxicology.

Cook GJ. 1994. Bacillus thuringiensis kurstaki exposure in ground-spray workers. Major Paper Submitted in Partial Fulfillment of MHSc Degree, (Community Medicine) in the Department of Health Care and Epidemiology, Faculty of Medicine, The University of British Columbia.

David R. 1990. Acute pulmonary toxicity/pathogenicity study of Dipel technical material. (*Bacillus thuringiensis* var. *kurstaki*) in rats: Lab Project No. G-7239. 001. Unpublished study prepared by Microbiological Associates Inc. 66 p. MRID 41443402.

Herms CP; Mccullough DG; Baue LS; Haack RA; Miller DL; Dubois NR. 1997. Susceptibility of the endangered Karner blue butterfly (Lepidoptera: lycaenidae) to *Bacillus thuringiensis* var. *kurstaki* used for gypsy moth suppression in Michigan. Great Lakes Entomol. 30:125-141

Hernandez E; Ramisse F; Cruel T; Vagueresse R; Cavallo JD. 1999. *Bacillus thuringiensis* serotype H34 isolated from human and insecticidal strains serotypes 3a3b and H14 can lead to death of immunocompetent mice after pulmonary infection. FEMS Immunol Med Microbiol. 24:43-47.

Hernandez E; Ramisse F; Gros P; Cavallo JD. 2000. Super-infection by *Bacillus thuringiensis* H34 or 3a3b can lead to death in mice infected with influenza A virus. FEMS Immunology Med Microbiol. 29:177-181.

Liebhold AM; McManus M. 1999. The evolving use of insecticides in gypsy moth management. J For. 97(3): 20-23.

Peacock JW; Schweitzer DF; Carter JL; Dubois NR. 1998. Laboratory assessment of the effects of *Bacillus thuringiensis* on native lepidoptera. Environ Entomol. 27: 450-457.

Petrie K; Thomas M; Broadbent E. 2003. Symptom complaints following aerial spraying with biological insecticide Foray 48B. New Zealand Medical Journal. 116: 1170-1177.

SERA 2004a. Control/Eradication Agents for the Gypsy Moth – Human Health and Ecological Risk Assessment for *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*) FINAL REPORT. SERA TR 04-43-05-02c dated June 8, 2004.

SERA 2004b. Control/Eradication Agents for the Gypsy Moth – Human Health and Ecological Risk Assessment for Gypchek – a Nuclear Polyhedrosis Virus (NPV) FINAL REPORT. SERA TR 04-43-05-02b dated June 16, 2004.

SERA 2004c. Control/Eradication Agents for the Gypsy Moth – Human Health and Ecological Risk Assessment for Diflubenzuron (Dimilin) FINAL REPORT. SERA TR 04-43-05-03b dated July 30, 2004.

SERA 2004d. Control/Eradication Agents for the Gypsy Moth – Human Health and Ecological Risk Assessment for Disparlure (a.i.) FINAL REPORT. SERA TR 04-43-05-04b dated August 27, 2004.

SERA 2004e. Control/Eradication Agents for the Gypsy Moth – Human Health and Ecological Risk Assessment for DDVP FINAL REPORT. SERA TR 05-43-05-5b dated August 20, 2004.

SERA 2004f. Control/Eradication Agents for the Gypsy Moth – Human Health and Ecological Risk Assessment for Tebufenozide (Mimic) FINAL REPORT. SERA TR 04-43-05-06c dated August 8, 2004.

SERA 2004g. Control/Eradication Agents for the Gypsy Moth – Human Health and Ecological Risk Assessment for the Gypsy Moth FINAL REPORT. SERA TR 04-43-05-07c dated August 23, 2004.

Stanton HC; Albert JR; Mersman HJ. 1979. Studies on the pharmacology and safety of dichlorvos in pigs and pregnant sows. Am J Vet Res. 40:315-320.

USDA (U.S. Department of Agriculture). 1995. Gypsy Moth Management in the United States: A Cooperative Approach. Final Environmental Impact Statement. Appendix F (Human Health Risk Assessment) and Appendix G (Ecological Risk Assessment).

U.S. EPA/OPP (U.S. Environmental Protection Agency/Office of Pesticide Programs). 1998. Reregistration Eligibility Decision (RED): *Bacillus thuringiensis*. Available at: http://cfpub.epa.gov/oppref/rereg/status.cfm?show=rereg

U.S. EPA/OPP (U.S. Environmental Protection Agency/Office of Pesticide Programs). 2004. Lepidopteran Pheromones Fact Sheet. Prepared by the Office of Pesticide Programs. Available at: http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/ factsheet_lep_pheromones.htm.

Tables

Table 2-1: Total use of control agents by numbers of acres treated between 1995 and 2003

	Suppression (Total acres)	Eradication (Total acres)	Slow-the-Spread (Total acres)	Grand Total (Acres)
B.t.k.	1,484,486	1,057,201	367,722	2,909,409
NPV	36,518	7,376	9,140	53,034
Diflubenzuron	657,671	6	6,883	664,560
Tebufenozide	0	0	0	0
Disparlure flakes	0	60,090	1,567,199	1,627,289
Mass Traps [*]	0	1,912	0	1,912

[*] Mass traps contain DDVP in a PVC strip and disparlure as an attractant.

Table 3-1: Comparative hazard identification for potential effects in humans

Endpoint	Gypsy Moth	*B.t.k.*	Agents used in Gypsy Moth Program LdNPV	DFB	Tebufen-ozide	DDVP	Dispar-lure
Lethality	○	□	○	□	□	●[a]	○
Sub-lethal effects							
Irritation	●	●	■	□	○	■	□
Blood				●	●	○[b]	
Carcinogenicity				■[c]	○	□	
Neurotoxicity		○	○	□	○	●	
Immunotoxicity		○	○	○	○	□	
Reproduction				○	■	■	
Endocrine Effects					○	□	
Pathogenicity		□ [d]	○	N/A	N/A	N/A	N/A

[a] Risks are mitigated by formulation in PVC.
[b] Excluding inhibition of plasma and RBC AChE.
[c] An environmental metabolite, 4-chloroanaline, poses a carcinogenic risk.
[d] *B.t.k.* itself does not appear to be pathogenic. Possible enhancement of influenza virus.

Key:		
	●	Effect/risk demonstrated in humans
	■	Effect is plausible
	□	Marginal evidence for potential effect
	○	No plausible basis for risk
	Blank	No data are available

Table 3-2: Comparative exposure assessment for human health effects

Agent	Measure of Exposure	Plausibility of Exposure	Comments
Gypsy moth	Eggs masses per acre	Variable	Exposure potential is high during outbreaks and decreases as intensity of infestation decreases.
B.t.k.	Application rate and cfu/m^3 x hour	High	During broadcast applications, exposure potential is high and can be reasonably well characterized.
LdNPV	mass of formulation	High	During broadcast applications, exposure potential is high and can be reasonably well characterized.
Diflubenzuron	mass of chemical	High	Can persist on vegetation and water contamination is plausible.
Tebufenozide	mass of chemical	High	Can persist on vegetation and water contamination is plausible.
DDVP	mass of chemical	Very low	Except in cases of intentional or incidental tampering with a trap, exposures will be very low.
Disparlure	mass of chemical	Variable	Very little compound is used in traps and exposures are likely to be very low.

Table 3-3: Comparative dose-response assessment for human health effects

Agent	Toxicity Value	Endpoint	Quality	Comment
Gypsy Moth	Acute	Irritation	●	Based on human data with a clear dose-response relationship.
B.t.k.	Acute	Irritation	■	Based on human data but no dose-response relationship is apparent.
		Toxicity	□	Based on a single study in mice using a marginally relevant route of exposure.
LdNPV	Acute	None	●	High confidence because no endpoint of concern can be identified.
Diflubenzuron	Acute	Blood	■	No EPA acute RfD. Conservative approach based on petroleum formulation.
	Chronic	Blood	●	Agency-wide EPA RfD adopted by OPP.
4-Chloroaniline[*]	Acute	Blood	■	No EPA acute RfD. Conservative approach based on 90-day study.
	Chronic	Blood	□	EPA chronic RfD. Confidence classified as low by EPA.
	Cancer Potency	Cancer	■	EPA cancer potency factor
Tebufenozide	Acute	Repro	■	No EPA acute RfD. Based on reproduction studies in two species
	Chronic	Blood	■	EPA/OPP chronic RFD.
DDVP	Acute	Neuro	●/□	For DDVP itself, value is based on an EPA acute RfD. For DDVP in PVC strip, the value is based on marginal data.
Disparlure	Acute	N/A	□	No acute RfD can be derived.

Key for quality of Toxicity Values:	●	High
	■	Medium
	□	Low

[*] An environmental metabolite of diflubenzuron.

Table 3-4: Comparative risk characterization for human health effects [a]

Agent	Hazard Quotient (HQ) [b]		Comments
	Lower	Upper	
Gypsy Moth	**1.6**	**625**	Irritant effects (dermal, ocular, and/or respiratory) are well documented. Lower range is based on sparse infestations, where effects might be seen in about 1% of the population. Upper range is based on major outbreaks where responses might be seen in about 40% of the population.
B.t.k.	0	0.04	HQs are for serious adverse effects, which are highly unlikely to occur. Irritant effects could be reported in about 20% of exposed individuals – both workers and members of the general public.
LdNPV	0	0.02	No risks are plausible. Upper range of HQ is calculated from a free-standing NOAEL.
Diflubenzuron			
Workers	0.05	0.5	The upper range is associated with the upper range of plausible exposures in ground spray applications. Under typical conditions, the HQ will be about 0.05.
Public	0.09	0.1	This narrow range of HQs reflects the higher HQ for any longer term exposure (0.09) and the highest HQ for acute exposures (0.1). Most other HQs are below 0.01.
4-Chloroaniline as an environmental metabolite of diflubenzuron			
Toxicity	0.02	0.4	Lower value is based on acute consumption of contaminated water (peak concentration) by child. Upper range based on acute consumption of contaminated fish by subsistence populations after accidental spill. Other HQs are insubstantial.
Cancer	0.09	0.4	HQs based on cancer risk of 1 in 1 million. Both lower and upper are based on consumption of contaminated water (central and upper ranges). Other scenarios lead to much lower risks.
Tebufenozide	0.03	**1.5**	Lower range is based on the central estimate of contaminated fruit (longer-term) after 2 applications. Highest HQ is for the upper range of longer-term consumption of contaminated fruit following 2 applications at the highest application rate. Other HQs are much less than 0.03.
DDVP	0	**380**	Lower range of risk is essentially zero because exposures are unlikely. Upper range is based on oral exposure from a child tampering with the strip. Likelihood of clinically significant effects seems remote.
Disparlure	0	0	No potential risk can be identified.

[a] See Figure 3-1 for illustration.
[b] Hazard quotients less than 0.01 are given as zero. For *B.t.k.*, the lower range of the HQ is 0.000036. For NPV and disparlure, risks are essentially zero. For DDVP, exposure is unlikely and the risk is also essentially zero except for accidental exposures.

Table 4-1: Comparative hazard identification for potential effects in nontarget species

Endpoint	Gypsy Moth	*B.t.k.*	LdNPV	DFB	Tebufen-ozide	DDVP	Dispar-lure
Terrestrial species							
Mammals	■	○	○	●	●	●	○
Birds	■	○	○	□	■	●	○
Nontarget *Lepidoptera*	■	●	○	●	●	●	■*
Other arthropods	□	○	○	●	●	●	○
Other invertebrates	□	□	○	○	○	●	○
Plants	●	○	○	○	○	○	
Microorganisms	■	○		□			
Aquatic species							
Fish	□	○	○	□	□	●	○
Invertebrates	□	■	○	●	●	●	■
Plants	□	○	○	□	■		
Microorganisms	■	○		□			

*Effects in other pest *Lepidoptera* pest species only.

Key:
- ● Direct effects demonstrated in species of concern
- ■ Effects are plausible
- □ Marginal evidence for effect
- ○ No plausible basis for risk
- Blank No data are available

Table 4-2: Comparative exposure assessment for ecological effects

Agent	Plausibility of Exposure	Primary Route	Comments
Gypsy moth	Variable	N/A	Exposure potential is high during outbreaks and decreases as intensity of infestation decreases.
B.t.k.	High	Oral	During broadcast applications, exposure potential is high.
LdNPV	High	Oral	During broadcast applications, exposure potential is high and can be reasonably well characterized.
Diflubenzuron	High	Oral	Can persist on vegetation and water contamination is plausible.
Tebufenozide	High	Oral	Can persist on vegetation and water contamination is plausible.
DDVP	Very low	Inhalation /Oral	Except in cases of insects entering the trap or other animals tampering with trap, exposures will be very low.
Disparlure	Variable	Variable	Very little compound is used in traps and exposures are likely to be very low.

Table 4-3: Comparative dose-response assessment for potential effects in nontarget species

Endpoint	Gypsy Moth	Agents used in Gypsy Moth Program						
		B.t.k.	Ld-NPV	DFB	4-CA	Tebufen-ozide	DDVP	Dispar-lure
Terrestrial species								
Mammals		■/□ [a]	□	■	■	■	■	
Birds		□		■	■	■		
Nontarget *Lepidoptera*	■	●		●		○		
Other arthropods				●		□		
Other invertebrates				□		○		
Plants	●							
Microorganisms				■				
Aquatic species								
Fish	□/○ [b]	□	□	○/■ [c]	□	■	■	■
Invertebrates		□	□	●	□	■	○	■
Plants				□	□	□		
Microorganisms				□	○			

[a] NOEC value only for oral exposure. NOEC and LOEC for inhalation.
[b] NOEC value only for tolerant species. LOEC only for sensitive species.
[c] Effect level only for acute exposures.

Descriptive Key:		
	●	Effect and no-effect levels clearly identified. Response or differences in sensitivities among species can be quantified.
	■	Effect and no-effect levels identified.
	□	Based on no-effect level only.
	○	Based on effect level only.
	Blank	No quantitative dose-response assessment is made.

Table 4-4: Comparative risk characterization for terrestrial species [a]

Agent	Hazard Quotient (HQ) [b]		Comments
	Lower	Upper	
Gypsy Moth	0.25	**400**	All HQs based on defoliation. Lower HQ based on low infestation (5 egg masses/acre) in intermediate stands. Upper HQ based on damage to sensitive stands in an outbreak (up to 83% defoliation). Effects secondary to defoliation will occur in some animal populations.
B.t.k.	0.36	**9.4**	All HQs based on lethality to terrestrial invertebrates using 10% as a benchmark. A maximum mortality of 3.6% for tolerant invertebrates and 94% for sensitive invertebrates
LdNPV	0	0	No toxicity to terrestrial species is likely. The upper range of the HQ is 0.001 and is based on the consumption of contaminated vegetation and an acute free-standing NOAEL in mammals.
Diflubenzuron	0.18	**32**	All HQs based on responses in terrestrial invertebrates. The lower range is based on tolerant species and the upper range on sensitive species.
4-Chloroaniline	0	0.02	The upper range based on the consumption of fish by a predatory bird after an accidental spill (acute scenario).
Tebufenozide	0	**4**	The upper range is based on the consumption of contaminated vegetation by a large mammal after 2 applications at the maximum application rate. While not quantified, effects on some nontarget *Lepidoptera* are possible.
DDVP	0	0	Typically, exposures will be minimal. Insects entering the traps are likely to be killed.
Disparlure	0	0	No potential hazard can be identified except possible mating disruption in other pest *Lepidoptera*.

[a] See Figure 4-1 for illustration. Note that the magnitude of the HQ among different agents is not a measure of relative risk or severity of effects. See text for discussion.
[b] Hazard quotients less than 0.01 are given as zero. For tebufenozide, the lower range of the HQ is 0.0002. For 4-chloroaniline the lower range of the HQ is 0.00002. For NPV and disparlure, lower range of the HQs are essentially zero. For DDVP, exposure is unlikely and the risk is also essentially zero except for accidental exposures.

Table 4-5: Comparative risk characterization for aquatic species [a]

Agent	Hazard Quotient (HQ) [b]			Comments
	Lower	Upper		
Gypsy Moth	0	0		No basis for asserting that adverse effects will be observed.
B.t.k.	0	0.5		All HQs based on aquatic invertebrates. Lower range is 0.007 for tolerant species. The upper range is based on sensitive species
LdNPV	0	0		No basis for asserting that adverse effects will be observed. The upper range is 0.00003 and is based on a free-standing NOEC.
Diflubenzuron	0	**5**		Upper range is based on acute effects in sensitive aquatic invertebrates (*Daphnia*) after peak exposures.
4-Chloroaniline	0	0.2		Upper range is based on acute exposures to aquatic invertebrates and aquatic plants.
Tebufenozide	0	0.4		Upper range is based on longer-term toxicity in sensitive aquatic invertebrates.
DDVP	0	0	**8**	No risks are plausible in normal use. The HQ for aquatic invertebrates could reach up to 8 in accidental exposures.
Disparlure	0	0.4		Upper range based on acute exposures to sensitive aquatic invertebrates (*Daphnia*).

[a] See Figure 4-1 for illustration. Note that the magnitude of the HQ among different agents is not a measure of relative risk or severity of effects. See text for discussion.
[b] Hazard quotients less than 0.01 are given as zero.

Figures

Figure 3-1: Risk comparison for potential human health effects.

Figure 4-1: Risk comparison for potential effects in terrestrial species.

Figure 4-2: Risk comparison for potential effects in aquatic species.

Pesticide Precautionary Statement

This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife--if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

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