Control/Eradication Agents for the Gypsy Moth - Human Health and Ecological Risk Assessment for Tebufenozide (Mimic) Final Report

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TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LIST OF APPENDICES

LIST OF TABLES

LIST OF FIGURES

NOTE: Tables followed by figures are placed after Section 5, References.

LIST OF WORKSHEETS

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AEL	adverse-effect level
a.i.	active ingredient
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 ($\mu g/cm^2$)	11.21
pounds per gallon (lb/gal)	grams per liter (g/L)	119.8
square centimeters (cm^2)	square inches (in^2)	0.155
square centimeters (cm^2)	square meters (m^2)	0.0001
square meters (m^2)	square centimeters (cm^2)	10,000
yards	meters	0.9144

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

CONVERSION OF SCIENTIFIC NOTATION

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

EXECUTIVE SUMMARY

OVERVIEW

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

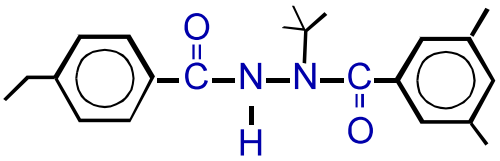
2.1. OVERVIEW

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 × 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.

3.1.3.1. 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 (≈ 12 mg/kg bw). At 2000 ppm (≈ 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:

$$1000 \text{ mg/kg/day} \times (1-e^{-0.00317\times6}) \times 5/7 = 13.45 \text{ mg/kg/day}.$$

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 × 0.07 mg/kg per ppm) and 70 mg/kg/day (LOAEL of 1000 ppm × 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

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).

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

– 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:

$$C_t = C_0 \times e^{-kt} \qquad \text{(Eq. 3-1)}$$

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 n^{th} application (C_n) can be calculated as:

$$C_n = C_0 \times (1 - p^n) \div (1 - p). \qquad \text{(Eq. 3-2)}$$

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:

$$C_{TWA} = (C_0 (1-\exp(-kt_1)) + [\{C_0 + C_0 \exp(-kt_1)\} \times \{1-\exp(-k [t_2 - t_1])\}]) \div (k t_2)$$

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).

3.3.4. 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 (≈160 mg/kg bw), with a NOAEL of 150 ppm (≈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 (≈ 12 mg/kg bw) tebufenozide; however, in the same study, rats given a dietary concentration of 2000 ppm (≈ 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.

4.3.3.2. 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.

4.3.3.3. 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.

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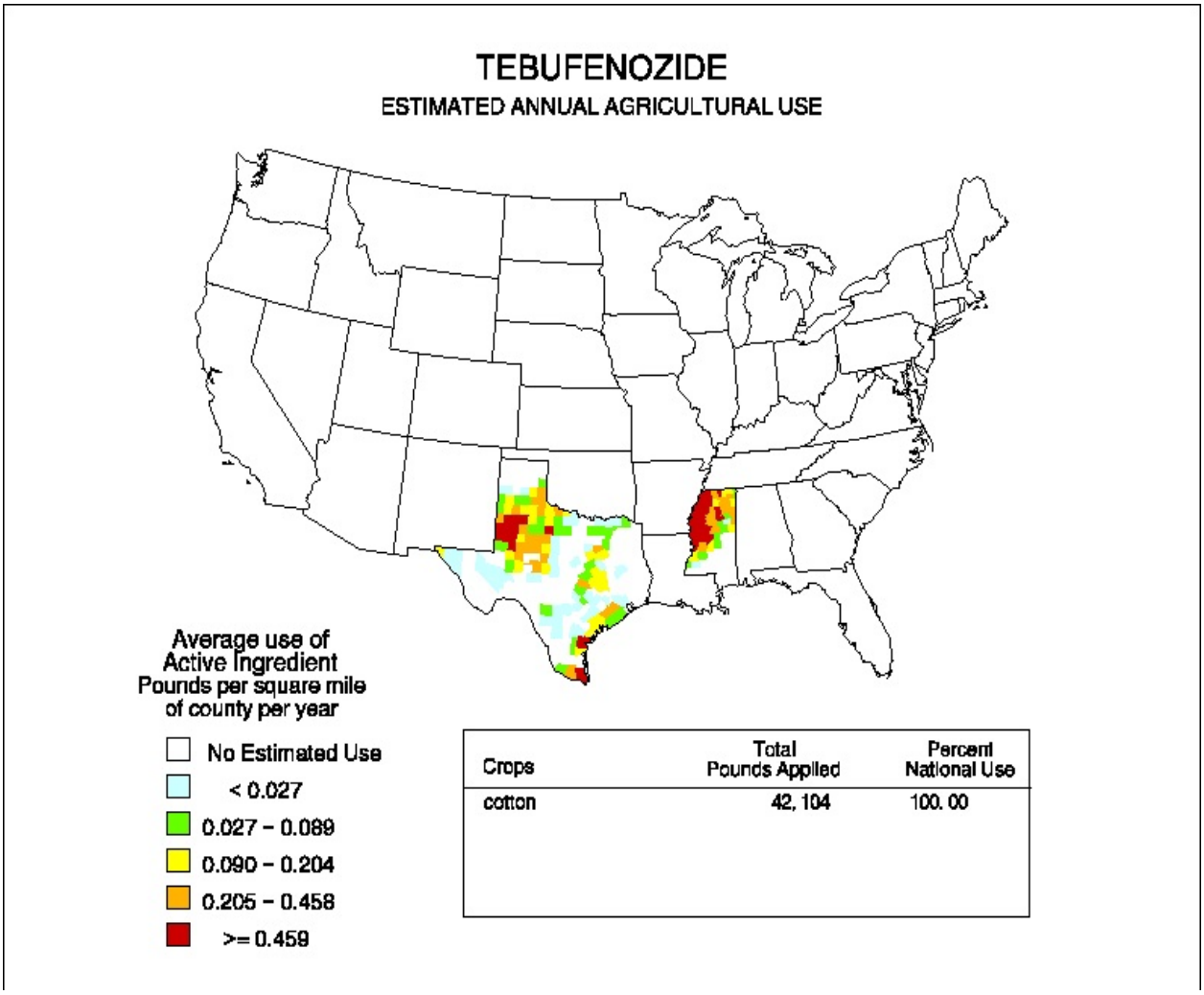

Figure 2-1: Agricultural Use of Tebufenozide on Cotton in 1992 (USGS 1998).

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		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	0.69713	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:

$$\log_{10} k_a = 0.233255 \log_{10}(\boldsymbol{k_{o/w}}) - 0.005657\, \boldsymbol{MW} - 1.49615$$

95% Confidence intervals for $\log_{10} k_a$

$$\log_{10} k_a \pm \boldsymbol{t}_{0.025} \times \boldsymbol{s} \times (\boldsymbol{a}'\boldsymbol{X}'\boldsymbol{X}\,\boldsymbol{a})^{0.5}$$

where $\boldsymbol{a}$ is a column vector of {1, MW, $\log_{10}(\boldsymbol{k_{o/w}})$}.

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 $\boldsymbol{a}$ because of the way the statistical analysis was conducted to derive X′X .

See following page for details of calculating $\boldsymbol{a}'\boldsymbol{X}'\boldsymbol{X}\,\boldsymbol{a}$ without using matrix arithmetic.

Worksheet A07a (continued)
Details of calculating a′X′X a

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

$$(\mathbf{X'X})^{-1} = \{ \{b\_1, b\_2, b\_3\}, \{c\_1, c\_2, c\_3\}, \{d\_1, d\_2, d\_3\} \},$$

$\mathbf{a'}\cdot(\mathbf{X'X})^{-1}\cdot\mathbf{a}$ is equal to

Term 1: {a_1 ×([a_1×b_1] + [a_2×c_1] + [a_3×d_1])} +
Term 2: {a_2 ×([a_1×b_2] + [a_2×c_2] + [a_3×d_2])} +
Term 3: {a_3 ×([a_1×b_3] + [a_2×c_3] + [a_3×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					
$\log_{10} k_a = 0.233255 \log_{10}(k_{o/w}) - 0.005657\ MW - 1.49615$			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	$hours^{-1}$					

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.

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 $\mathbf{a}' \cdot (\mathbf{X}'\mathbf{X})^{-1} \cdot \mathbf{a}$ - see Worksheet A07b for details of calculation.								
Term 1	-0.0220577884							
Term 2	0.007751756							
Term 3	0.0564889197							
$\mathbf{a}' \cdot (\mathbf{X}'\mathbf{X})^{-1} \cdot \mathbf{a}$	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	±	$t_{0.025}$	×	s	×	$\mathbf{a}' \cdot (\mathbf{X}'\mathbf{X})^{-1} \cdot \mathbf{a}^{0.5}$	
Lower limit	-2.18337858572	-	1.9600	×	0.727129	×	0.2054263858	
Upper limit	-1.59784237428	+	1.9600	×	0.727129	×	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: 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 ($p \leq 0.05$) and relative spleen weight was 44% greater ($p \leq 0.01$); in females there was a significant dose response in relative spleen weight ($p \leq 0.05$); 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.			
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: 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 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.			
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: 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			
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: 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			
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: 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/Toxi-city 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: 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: 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			
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: 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 ≥ 24 hours but ≤7 days.	Gingrich and Parno 1994s MRID 444727706

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: 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.

Supplemental Tables for Appendix 4

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: <u>1996 season</u> 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 <u>1997 season</u> 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	<u>For the 1996 season</u> Cumulative mortality as follows: low dose: 34.4% high dose: 39.4% untreated control: 0% <u>For the 1997 season</u> 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.

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)	<u>Cuticle penetration</u>: DFB 16% TEB 26% <u>Excretion</u>: 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 µg/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 µg/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. 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

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). Recently emerged moths exposed to treated surfaces (360 ppm*) for 24 hours, then mated with unexposed partner (oviposit on non-treated surface) tebufenozide was RH-5992, 2F (flowable) * authors state that this is the recommended field rate	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%). 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	Sun and Barrett 1999
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". Difubenzuron (DFB) "Dimilin 25W at 237 mL per 378 L final solution, without added sticker".	Laboratory-reared gypsy moth larvae (1^{st}, 2^{nd}, 3rs, and 4^{th} 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. 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.	Webb et al. 1998

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 ≥1.5 ppm 3rd instar: no survival to pupation at concentrations ≥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 in1st 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			
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 (≥ 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.			
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	<u>One higher dose application</u>: • 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%). <u>Two lower dose applications</u>: • 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 ≤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 ≤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: 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			
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: Toxicity of tebufenozide to aquatic invertebrates and algae.

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 ≥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