VERBENONE - Human Health and Ecological Risk Assessment FINAL REPORT

Prepared for:

USDA, Forest Service

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Submitted to:
Leslie Rubin, COTR
Animal and Plant Health Inspection Service (APHIS)
Biotechnology, Biologics and Environmental Protection
Environmental Analysis and Documentation
United States Department of Agriculture
Suite 5A44, Unit 149
4700 River Road
Riverdale, MD 20737

Submitted by:
Syracuse Environmental Research Associates, Inc.
5100 Highbridge St., 42C
Fayetteville, New York 13066-0950
Telephone: (315) 637-9560
Fax: (315) 637-0445
E-Mail: SERA_INC@msn.com
Home Page: www.sera-inc.com

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

a.e.	acid equivalents
a.i.	active ingredient
AEL	adverse-effect level
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
bw	body weight
cm	centimeter
EC_{50}	concentration causing 50% inhibition of a process
EC_{100}	concentration causing complete inhibition of a process
EIS	environmental impact statement
F	female
F_1	first filial generation
FS	Forest Service
g	gram
GC	gas chromatography
GRAS	generally recognized as safe
HQ	hazard quotient
IARC	International Agency for Research on Cancer
i.p.	intraperitoneal
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
K_p	skin permeability coefficient
L	liter
lb	pound
LC_{50}	lethal concentration, 50% mortality
LD_{50}	lethal dose, 50% mortality
LD_{95}	lethal dose, 95% mortality
LOAEL	lowest-observed-adverse-effect level
m	meter
M	male
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
MW	molecular weight
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NRC	National Research Council
ppm	parts per million

ACRONYMS, ABBREVIATIONS, AND SYMBOLS (*continued*)

RBC	red blood cells
RfD	reference dose
UF	uncertainty factor
U.S.	United States
U.S. EPA	U.S. Environmental Protection Agency
USDA	United States Department of Agriculture
>	greater than
≥	greater than or equal to
<	less than
≤	less than or equal to
=	equal to
≃	approximately equal to

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

To convert …	Into …	Multiply by …
acres	hectares (ha)	0.4047
acres	square meters (m^2)	4,047
atmospheres	millimeters of mercury	760
centigrade	Fahrenheit	$1.8C^\circ + 32$
centimeters	inches	0.3937
cubic meters (m^3)	liters (L)	1,000
Fahrenheit	centigrade	$0.556F^\circ$-17.8
feet per second (ft/sec)	miles/hour (mi/hr)	0.6818
gallons (gal)	liters (L)	3.785
gallons per acre (gal/acre)	liters per hectare (L/ha)	9.34
grams (g)	ounces, (oz)	0.03527
grams (g)	pounds, (oz)	0.002205
hectares (ha)	acres	2.471
hectares (ha)	square meters	10,000
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

Introduction

Verbenone is an antiaggregation pheromone that will be used by the Forest Service for the control of southern pine beetle infestations. In other words, verbenone is a compound that can be used to disrupt the behavior of some forest insect pests in a manner that inhibits infestations. The U.S. EPA recently registered verbenone as an insect control agent. The human health and ecological risk assessments in this document were prepared to support an appraisal of the environmental consequences of using verbenone in Forest Service programs.

The overriding factor in both the human health and ecological risk assessments is uncertainty associated with the very limited data on the potential adverse effects of verbenone. There are no chronic studies regarding any species after exposure to verbenone. Furthermore, verbenone was not tested for carcinogenicity and reproductive effects. Consequently, the limited data severely constrains the conclusions that can be drawn regarding the potential effects of verbenone to either humans or wildlife species.

Program Description

All three formulations of verbenone, bubble caps, beads, and pouches are designed to release verbenone at a slow and relatively constant rate for a prolonged period of time. Presently, the Forest Service anticipates using only the pouch formulation of verbenone in its programs. Pouch formulations contain 4.65 g of verbenone that is released over a 20- to 40-day period. The inert ingredients include a release pouch and an absorbent reservoir. Verbenone pouches are affixed to infested trees using a special device to nail the pouch at approximately 15 feet above the ground surface. Depending on the severity of the infestation, one to nine pouches may be applied per infested tree.

HUMAN HEALTH RISK ASSESSMENT

Hazard Identification

There is only limited data regarding the toxicity of verbenone to experimental mammals. Specifically, no information is available on the chronic toxicity, reproductive effects, or carcinogenicity of this compound. Typically, the U.S. EPA waives a number of standard mammalian toxicity tests for pheromones in the registration process. The agency bases its decision on the expectation that the potential for adverse effects as a result of exposure to pheromones is relatively low because the pheromones will be used in relatively small amounts and are generally not toxic to nontarget species. As a consequence of the decision, the amount of toxicity data on verbenone in U.S. EPA's FIFRA CBI studies is minimal. The only other available information regarding the potential toxicity of verbenone to experimental mammals comes from a series of three short studies published in the Italian literature in 1986. These studies investigate the potential mechanisms for the reported anti-inflammatory activity of verbenone. Verbenone was not tested for carcinogenicity, mutagenicity, or reproductive effects.

Exposure Assessment
Under typical conditions of placing the verbenone pouch, workers should not be exposed to substantial levels of verbenone. The verbenone is encased in a sponge matrix that releases only small quantities of verbenone per unit time. Without violating the proprietary nature of the packaging material, it can be disclosed that the entire verbenone sponge is encased in material that is impermeable to verbenone. Were this not true, the pouch formulation would have an unacceptably short shelf life.

There are no studies regarding worker exposure to verbenone. Although the default methods for estimating worker exposure are likely to overestimate absorbed doses, they are applied in the absence of other methods to estimate exposure. Using these methods results in estimated absorbed doses of 0.08 (0.008-0.3) mg/kg/day from routine applications of verbenone.

Two accidental exposure scenarios are also considered, including inadvertent dermal contact during placement of the verbenone pouch by workers and imprudent handling of the pouch by a child. The worker scenario postulates that the pouch is ruptured in some way during its removal from the packaging material or during its placement on a tree. Based on the assumption that both hands are contaminated with verbenone for 1 minute, the estimated absorbed dose is 1.5 (0.97-2.1) mg/kg bw. In a worst case and perhaps highly implausible exposure scenario, in which the worker does not clean the contaminated hands for 1 hour, exposure will not exceed 63.7 mg/kg. In this case, the dose estimate is limited by the amount of verbenone contained in a single pouch.

Like workers, members of the general public are not likely to be exposed to substantial levels of verbenone. Nonetheless, as a worst case scenario, it is assumed that a child could encounter a pouch that was accidently dropped on to the ground or removed in some way from a tree. In this scenario, both dermal and oral exposure could occur through imprudent handling of the bubble cap. The estimated absorbed doses for the scenario range from 35 to 350 mg/kg bw.

Dose-Response Assessment
Except for standard acute toxicity studies and some reports from the Italian literature on the anti-inflammatory activity of verbenone, there is no information on dose-response relationships for verbenone in humans or experimental mammals. Acute doses as low as about 1000 mg/kg could be expected to cause mortality in rats, and a NOAEL of 60 mg/kg is reported for acute anti-inflammatory activity.

Risk Characterization
Risks are typically characterized quantitatively as a hazard quotient, which is an estimated level of exposure divided by some expression of an acceptable level of exposure. For verbenone, quantitative risk characterizations are not justified because of the very limited data available on the toxicity of this compound as well as the uncertainties in the estimated levels of exposure.

Notwithstanding these limitations, there is no indication that toxicologically significant exposures to verbenone are plausible under typical conditions of use for either workers or members of the general public. The information that is available suggests that implausibly high estimates of worker exposure are below the most sensitive endpoint known for verbenone, which is a NOAEL of 60 mg/kg for acute anti-inflammatory activity in rats. Accidental exposures for workers could result in absorbed doses that might reach or slightly exceed the NOAEL in the rat study. Extreme accidental exposure scenarios for the general public—specifically a small child ingesting all of the verbenone in a single verbenone pouch—result in estimates of absorbed dose that approach the LD_{50} in rats.

An assessment of the potential consequences of longer-term exposure to verbenone cannot be made from the available data. Verbenone is a naturally occurring substance, which has no impact on the characterization of risk. Although many naturally occurring substances are benign or even essential, others can be extremely harmful.

Accordingly, this risk assessment for potential human health effects is dominated by uncertainty (i.e., a lack of knowledge). It is not possible to completely characterize the potential effects in humans after exposure to verbenone used in Forest Service programs, given the limited information that is available regarding the toxicity of the compound..

ECOLOGICAL RISK ASSESSMENT

Hazard Identification

Like the human health risk assessment, the ecological risk assessment is governed by the limitations of the toxicity data on verbenone. The acute toxicity of verbenone was assayed in rats, bobwhite quail, three species of terrestrial invertebrates, two fish species, and one aquatic invertebrate. There are few additional data on verbenone that impact this risk assessment. As in the case of the human health risk assessment, the lack of information regarding the toxicity of verbenone is mitigated somewhat by the limited use proposed by the Forest Service.

Exposure Assessment

The only exposure scenarios developed for quantitative use in the risk characterization involve terrestrial animals consuming verbenone from a pouch formulation. This scenario is plausible, given that the placement of verbenone pouches is likely to make them available to terrestrial species.

Quantitative exposure scenarios for aquatic species are not plausible. Nonetheless, the extremely low levels of verbenone that might be found in water are taken into consideration.

Dose-Response Assessment

Dose-response relationships for acute toxic effects are available in rats, bobwhite quail, three species of nontarget insects, and three aquatic animals. Gavage administration of 1500 mg/kg verbenone caused mortality in rats. An acute study on anti-inflammatory activity reports a NOAEL of 60 mg/kg and an effect level of 120 mg/kg. The study in birds (bobwhite quail)

reports a NOAEL of 300 mg/kg/day based on the absence of overt signs of toxicity and changes in body weight. In aquatic species, the lowest reported 96-hour LC_{50} value is 130 (100-160) mg/L (trout). Given the proposed use of verbenone and the improbability of ambient water becoming contaminated with verbenone, these data are adequate for risk characterization.

There is only one publication regarding the toxicity of verbenone to nontarget terrestrial insects. Verbenone can cause mortality after either direct contact (176 µg/fly) or vapor (7-22 $\mu g/cm^3$) exposures. In addition, soil concentrations of about 50 ppm cause mortality in about 50% of southern corn rootworm larvae, and liquid media concentrations of 833 ppm caused a 65% inhibition of housefly egg hatch.

There are no data in the literature regarding the toxicity of verbenone to terrestrial or aquatic plants or microorganisms.

Risk-Characterization

The overriding factor in the ecological risk assessment is the paucity of information on the toxicity of verbenone. Data regarding chronic toxicity or potential reproductive effects after exposure to verbenone are not available. Furthermore, the acute toxicity of verbenone was assayed in only a few species. Thus, the characterization of risk is limited. There is no evidence to suggest that exposure to verbenone will have a substantial impact on any species populations; nevertheless, exposure to toxic levels may affect individual organisms under conditions of extreme exposure.

Given the limited use of verbenone proposed by the Forest Service, two exposure scenarios are plausible: tampering with a bubble cap and exposure to very low levels of verbenone in the air. If an animal were to tamper with a verbenone pouch, the amount of verbenone that might be consumed or otherwise absorbed could range from negligible to the total amount of verbenone in the pouch, which is about 4650 mg. The consequences of such an event will vary depending on the size of the animal. A number of small to medium sized mammals ranging in body weight from 20 to 3000 g could be exposed to lethal doses of verbenone. Larger mammals are less likely to be exposed to a lethal dose—provided that they tampered with only one verbenone pouch—but could be exposed to levels greater than the threshold for anti-inflammatory activity. It is unclear whether such exposures would cause adverse effects. If birds are as sensitive as mammals to verbenone, a number of birds also might be exposed to lethal doses of verbenone if they were to tamper with the verbenone pouch.

The probability of wildlife species consuming lethal amounts of verbenone cannot be assessed from the available data. What is clear is that the pouches would be available. Nonetheless, the efficacy of verbenone was investigated in field studies, and if wildlife species commonly sought and consumed verbenone, the event would probably be reported. Thus, despite the potential risk to an individual animal, it seems unlikely that the consumption of verbenone by wildlife will have a detectable or substantial impact on the population of any species.

Many species will be exposed to low levels of verbenone in air because of the proposed way in which the compound will be used by the Forest Service. The available data are not useful for assessing the consequences of inhalation exposure to verbenone for mammals and birds. Moreover, there are no data regarding ambient air concentrations of verbenone either from natural sources or from the application of verbenone in the field. Consequently, the risk of inhalation exposure to verbenone cannot be characterized quantitatively. Nonetheless, a very crude comparison made in the human health risk assessment of possible verbenone concentrations in air to estimates of verbenone that might be absorbed suggests that toxicologically significant levels of exposure are implausible. This conclusion is reinforced by data on insects that suggest that exposure levels in air are likely to be from 35,000 to 70,000 times lower than lethal concentrations.

The exposure of aquatic species to substantial quantities of verbenone is implausible. Based on the available acute toxicity data, there is no evidence to suggest that even extreme accidental exposures would have an impact on aquatic species.

1. INTRODUCTION

Verbenone is an antiaggregation pheromone that will be used by the Forest Service for the control of southern pine beetle infestations. In other words, verbenone is a compound that can be used to disrupt the behavior of some forest insect pests in a manner that inhibits infestations. In addition, verbenone is a naturally occurring constituent of various species of pine trees and is produced by some microorganisms. The U.S. EPA recently registered verbenone as an insect control agent. The human health and ecological risk assessments in this document were prepared to support an appraisal of the environmental consequences of using verbenone in USDA Forest Service programs.

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 verbenone, 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 1998).

There is very little information regarding the toxicology of verbenone. Most of the available toxicology studies are unpublished and were submitted to the U.S. EPA in support of the registration of verbenone. Given the preponderance of unpublished relevant data in U.S. EPA files and the lack of a data review on verbenone, SERA, Inc conducted a complete search of the U.S. EPA files during the preparation of this document. Full-text copies of all studies submitted to the U.S. EPA were kindly provided by the U.S. EPA Office of Pesticide Programs. These studies were reviewed, and synopses of the most relevant studies are provided in the appendices to this document. The Forest Service provided SERA, Inc with a copy of the experimental use permit submissions for verbenone (Phero Tech 1988), which for the most part are identical to the studies submitted to the U.S. EPA in the registration package.

In general, the risk assessment methods used in this document are similar to those used in risk assessments conducted previously by the Forest Service as well as in risk assessments conducted by other government agencies. Details regarding the specific health risk assessment methods used in this document are provided in SERA (1998). More detailed explanations of specific methods used to estimate occupational exposure are provided in Rubin et al. (1998). Similar documentation for methods of assessing dermal absorption is provided in Durkin et al. (1998).

Risk assessments are usually expressed with numbers; however, the numbers are far from exact. *Variability* and *uncertainty* may be dominant factors in any risk assessment and these factors should be expressed. Within the context of a risk assessment, the terms *variability* and *uncertainty* signify different conditions.

Variability reflects the knowledge of how things may change. Variability may take several forms. For this risk assessment, three types of variability are distinguished: *statistical*, *situational*, and *arbitrary*. *Statistical variability* reflects at least apparently random patterns in data. For example, various types of estimates used in this risk assessment involve relationships of certain physical properties to certain biological properties. In such cases, best or maximum likelihood estimates can be calculated as well as upper and lower confidence intervals that reflect the statistical variability in the relationships. *Situational variability* describes variations depending on known circumstances. For example, the application rate or the applied concentration of a herbicide will vary according to local conditions and goals. As discussed in the following section, the limits on this variability are known and there is some information to indicate what the variations are. In other words, *situational variability* is not random. *Arbitrary variability*, as the name implies, represents an attempt to describe changes that cannot be characterized statistically or by a given set of conditions that can be adequately described. This type of variability dominates certain spill scenarios involving either a chemical spilled onto the surface of the skin or spilled into water. In either case, exposure depends on the amount of chemical spilled and the area of skin or volume of water that is contaminated.

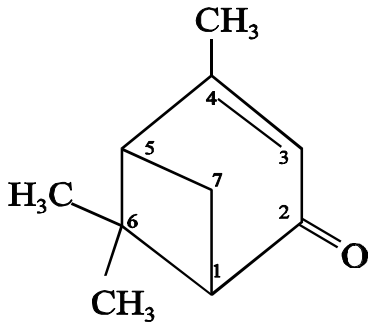
Uncertainty reflects a lack of knowledge. For example, the focus of the human health dose-response assessment is to estimate an “acceptable” or “no adverse effect“ dose level for human exposure. For verbenone and for most other chemicals, however, this estimate for humans must be based on data from studies on experimental mammals, which cover only a limited number of effects. The methods used for making this assessment are, for the most part, based on judgment rather than analytical methods. Although the judgments may reflect a consensus (i.e., be used by many groups in a reasonably consistent manner), the resulting estimates cannot be proven analytically. In other words, the estimates regarding risk involve uncertainty.

For verbenone specifically, the overriding factor in both the human health and ecological risk assessments is uncertainty associated with the very limited data on the potential adverse effects of verbenone. There are no chronic studies on the toxicity of verbenone to any species and verbenone has not been tested for carcinogenicity and reproductive effects. Consequently, conclusions that can be drawn on the potential effects of verbenone to either humans or wildlife species are extremely limited.

2. PROGRAM DESCRIPTION

2.1. CHEMICAL DESCRIPTION AND COMMERCIAL FORMULATIONS

Verbenone is the common name for (1R-cis)-4,6,6-trimethylbicyclo-[3.1.1]hept-3-en-2-one:



Selected chemical and physical properties of verbenone are summarized in Table 2-1. The information on verbenone that is quantitatively used in this risk assessment is summarized in worksheet B01. As summarized in Table 2-1, the nomenclature for verbenone is somewhat complex because the compound can exist in two enantiomers (i.e., mirror images that cannot be - superimposed) as well as in differing mixtures of these enantiomers.

Verbenone is a pheromone, a naturally occurring chemical involved in the transmission of messages (i.e., chemical communication) within or among species. Some pheromones are attractants and may be used in the trapping of pest species. Others, like verbenone, have the opposite effect, causing the organism to be repelled or dispersed. The chief characteristics of pheromones are that they are effective at very low concentrations and are specific either to an individual species or groups of related species (Vite and Baader 1990). In addition to being synthesized by insects (section 4.1.2.3), verbenone occurs naturally through the autooxidation of á-pinene, a chemical commonly found in the resin of many species of pine (Hunt et al. 1989).

The Forest Service anticipates using verbenone as a pheromone to disrupt infestations of the mountain pine beetle (Hunt and Borden 1990; Lindgren and Borden 1993; McGregor. 1988a,b; Shore et al. 1992), southern pine beetle (Burke and Lindgrem 1993; Grosman et al. 1997; Salom et al. 1992), and western pine beetle (McGregor 1988c). Verbenone was evaluated also as a control for the pales weevil (Salom et al. 1994); however, this use does not appear to be currently proposed or registered.

There are three available formulations of verbenone: bubble caps, beads, and pouches, all of which are designed to release the compound at a slow and relatively constant rate over a prolonged period of time. The bubble cap formulation contains 0.49 g of verbenone with a stabilizer (high molecular weight phenol antioxidant), an inert release membrane, and an inert cavity with a total weight of 1.275 g.(LaFontaine and Williamson 1988a). Verbenone beads contain 5.45 g of verbenone, 0.005 g of a stabilizer (Ethanox 330), and an inert bead 448.55 g (LaFontaine and Williamson 1988b). In a letter to U.S. EPA requesting an extension of an experimental use

permit, Phero Tech indicated that the formulation was going to change from a 1% active to a 1.2% active 'load' (Burke. 1993). The specification given by LaFontaine and Williamson (1988b) is consistent with a 1.2% load (i.e., 5.45 g of verbenone / 5.45 g of verbenone + 448.55 g inert bead = 0.012 or 1.2%). Some bead formulations may also contain Cyasorb UV-5411 (2-(2-hydroxy-5-tert-octylphenyl) benzotriazole, CAS 3147-75-9) (LaFontaine and Williamson 1996).

Pouch formulations are reported to contain 4.65 g of verbenone and release 260 mg/day at 30°C. Under field conditions, the anticipated field life is 20- to 40-days depending on the weather (LaFontaine and Williamson 1996). This corresponds to release rates of about 115 to 230 mg/day [4,650 mg ÷ 20 to 40 days = 116.25 to 232.5 mg/day]. A recent product label for the pouch formulation indicates that it contains 4.46 g of verbenone, 0.14 g of related compounds, and 0.05 g of a stabilizer (Phero Tech 2000). These materials [a total of 4.65 g] are classified as active ingredients on the current product label. Currently, the pouches contain a sponge material that holds the verbenone. The inert ingredients, which include a release pouch and absorbent (pouch) reservoir, account for 26% of the total product weight. Thus, the total weight of the pouch formulation is 6.28 g [4.65g÷(1-0.26)].

These formulations were developed by Phero Tech Inc, and the formulation specifications were submitted to the U.S. EPA. Specific information regarding the formulation is proprietary under FIFRA Section 10(d)(1)(A). The proprietary information was submitted to the U.S. EPA as FIFRA CBI (Confidential Business Information) (LaFontaine and Williamson 1988a,b,c,d; LaFontaine and Williamson 1996; Phero Tech Inc.1993a,b; Phero Tech 1996). Although the information from these CBI submissions was reviewed in the preparation of this risk assessment, the information cannot be discussed in this risk assessment except to state that the CBI data do not have a substantial impact on the risk assessment.

At this time, the Forest Service anticipates using only the pouch formulation of verbenone.

2.2. APPLICATION METHODS AND RATES

Because verbenone is not currently used by the Forest Service except in experimental applications, there are no standards regarding application rates or methods. In the application for experimental use submitted to the U.S. EPA (Phero Tech 1988), application rates ranging from 3 to 5 lbs/acre were recommended for the bead formulation, which may be applied aerially. The Forest Service evaluated the bead formulation (Shea et al. 1992) but does not anticipate its use or aerial application.

For the pouch formulation, verbenone will be applied only to infested trees in a relatively small localized area. Within the infested area, verbenone pouches will be applied at rates of 25 to 40 mL per square foot of infested trees (Clarke et al. 1999). In infestations, 120 square feet of trees will typically be infested per acre (43560 sq. ft.) (Clark 2000) for a ratio of about 0.003 [120÷43560 = 0.002755]. Using this ratio and a specific gravity of 0.97 g/mL (LaFontaine and Williamson 1988a), this corresponds to an application rate of about 0.8 to 1.2 kg/ha,

$$0.003 \times (25 \text{ to } 40 \text{ mL} \times 0.97 \text{ g/mL} \div \text{ft}^2) \times 10{,}760 \text{ ft}^2/\text{ha} \div 1000 \text{ g/kg} = 0.783 \text{ to } 1.252 \text{ kg/ha},$$

or about 0.7 to 1.1 lbs/acre. Because verbenone is only applied to relatively small infested area, these application rates are not comparable to those of compounds that may be broadcast over wide areas.

As specified on the current product label, the verbenone pouches are affixed to infested trees. A special device, referred to as a Hundle hammer is used to nail the pouch to a tree at about 15 feet above the ground surface. The number of pouches that are applied to each tree will vary with the size of the tree and the severity of the infestation (Clarke 1999). The current product label specifies using from 1 to 6 pouches per tree. Supplemental use directions that accompany the label as well as the recent publication by Clarke (1999) specify up to 9 pouches per infested tree.

An individual worker may treat from 4 to 5 infestations per day. The number of pouches used per infestation will vary with number of infested trees as well as the treatment technique—verbenone only or verbenone plus felling. Overall, it is anticipated that a worker could apply between from 100 to 700 pouches per infestation with a central estimate of about 400 pouches per infestation. Thus, the lower range for the number of pouches handled per day by a worker may be estimated at 400 pouches per day [100 pouches per infestation × 4 infestations per day]. An upper range of 3500 pouches per day [700 pouches per infestation × 5 infestations per day], however, is implausible and a more reasonable upper estimate is judgmentally set at 2500 (Clarke 2000).

Table 2-1. Selected physical and chemical properties of verbenone.

Synonyms/Nomenclature	2-pinen-4-one; (Budavari 1989) 2-pinene-4-one (LaFontaine and Williamson 1996) levorotatory enantiomer: [s]-verbenone (-)-verbenone (1S)-4,6,6-trimethylbicyclo-[3.1.1]hept-3-en-2-one; d-verbenone dextrorotatory enantiomer: [r]-verbenone (+)-verbenone (1R)-4,6,6-trimethylbicyclo-[3.1.1]hept-3-en-2-one; d-verbenone
CAS Number	18309-32-5 (dextrorotatory) 1196-01-6 (levorotatory) 80-57-9 (racemic mixture)
Molecular weight	150.21 (Budavari 1989)
Natural sources	auto-oxidation of á-pinene (Hunt et al. 1989) verbena oil from *Verbena triphiylla* (Budavari 1989) frass of some pine beetles (Gregoire et al. 1991) southern pine beetle (Grosman et al. 1997)
Appearance, ambient	light yellow liquid with a pine odor (LaFontaine and Williamson 1988a,b)
Solubility	Practically insoluble in water. Miscible in all proportions with the many organic solvents. (Budavari 1989)
K_{ow}	144.9 (LaFontaine and Williamson 1988a)
Specific Gravity (water = 1)	0.97 (LaFontaine and Williamson 1988a)
Persistence in air	rapidly (t_{50} of 75 to 100 hours) converted to chrysanthenone in sunlight by photoisomerization.

3. HUMAN HEALTH RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION

3.1.1. Overview. There is not much information regarding the toxicity of verbenone to experimental mammals. Specifically, no information is available on the chronic toxicity, reproductive effects, or carcinogenicity of this compound. The U.S. EPA usually waives a number of standard mammalian toxicity tests for pheromones in the registration process. The decision to do so is based on the assumption that since pheromones are commonly used in relatively small amounts and are generally not toxic to nontarget species, the potential for adverse health effects is relatively low. Consequently, the U.S. EPA's FIFRA CBI studies do not contain a lot information about the toxicity of verbenone. The only other source of information regarding the potential toxicity of verbenone to experimental mammals is a series of three short studies published in the Italian literature in 1986. The studies investigate the potential mechanisms for the reported anti-inflammatory activity of verbenone.

3.1.2. Acute Toxicity. The CBI studies were reviewed, and the information that can be disclosed is summarized in Appendix 1. The CBI files contain only one study pertaining to the acute oral toxicity of verbenone (Deenihan 1987a,b,c). This study appears to have been submitted to the U.S. EPA on three separate occasions, probably in support of each of the three verbenone formulations discussed in section 2. After male and female Sprague-Dawley rats were administered single gavage doses of verbenone in a vegetable oil, the estimated LD_{50} values (with 95% confidence intervals) were 3400 (2833 to 4080) mg/kg for males and 1800 (1000 to 3240) mg/kg for females. Of the animals that died after dosing, all but one death occurred within 24 hours of dosing. The primary overt signs of toxicity were tremors and convulsions. Additional signs of toxicity included weight loss and lethargy. Gross pathological changes included mottled lungs, "green material and gas in some of the animals" intestines, mottled liver, reddened adrenals. No mortality and no gross pathological changes were observed in any of the control animals.

The potential toxicity of verbenone to experimental mammals is addressed also in the Italian literature, which includes a series of three short studies published in 1986 (Chiariello et al. 1986a,b,c) that invesitagate the potential mechanisms for the anti-inflammatory activity of verbenone reported by Folisi et al. (1982). Chiariello et al. (1986a) report that verbenone inhibited the activity of arachidonic acid (an essential fatty acid that has anti-inflammatory activity), ADP, and thrombin on *in vitro* blood platelet aggregation. Inhibition of ADP and thrombin activity had apparent thresholds of 100 and 1000 mg verbenone/L blood, respectively. There was no apparent threshold for the inhibition of arachidonic acid activity. At the lowest concentration of verbenone tested, 1 mg/L blood, platelet aggregation was inhibited by 3% (Chiariello et al. 1986a, Table 1, page 388). The studies by Chiariello et al. (1986b,c) demonstrate that verbenone has pharmacological activity similar to that of indomethacin, a standard anti-inflammatory drug that blocks prostaglandin biosynthesis (Budavari 1989), after either intravenous (Chiariello et al. 1986b) or oral (Chiariello et al.1986c) exposure. In the oral study, the apparent threshold for verbenone in rats after gavage administration was 60 mg/kg with an effect level of 120 mg/kg (Chiariello et al. 1986c).

3.1.3. Subchronic or Chronic Systemic Toxic Effects. There is no information regarding the subchronic or chronic toxicity of verbenone in the published literature or in the U.S. EPA FIFRA/CBI files.

3.1.4. Reproductive and Teratogenic Effects. There is no information regarding the potential reproductive or teratogenic effects of verbenone in the published literature or in the U.S. EPA FIFRA/CBI files.

3.1.5. Carcinogenicity and Mutagenicity. There is no information regarding the potential carcinogenic or mutagenic effects of verbenone in the published literature or in the U.S. EPA FIFRA/CBI files.

3.1.6. Effects on the Skin and Eyes. Verbenone was tested for acute dermal toxicity, dermal irritation, and ocular damage (Deenihan 1987a,b,c). As noted in section 3.1.2, the reference to Deenihan (1987a,b,c) designates a single report that appears to have been submitted to the U.S. EPA on three separate occasions. Details of the studies from this report are summarized in Appendix 1.

In the dermal toxicity study, 2000 mg/kg of undiluted verbenone was applied to the abraded skin of rabbits, covering about 10% of body surface. The treated area was occluded for 24 hours after which time the residual test material was removed. No mortality occurred in any of the treated animals over a 14 day observation period. All five treated male rabbits and three of five treated female rabbits exhibited mild irritation in areas receiving test material. These responses were classified using Draize scores of 1-2 for erythema and edema. Several days after treatment, sloughing of skin was observed at the test site in some animals. No abnormalities in internal organs were observed on necropsy.

In the dermal irritation study, 0.5 g of verbenone was applied to the intact skin of six rabbits, and the treated area was covered with one inch square gauze patches. The entire trunk of each animal was then wrapped for 4 hours, after which residual material was removed by washing. Observations included slight erythema in two of six treated animals and mild edema in one of six treated animals.

In the eye irritation study, 0.1 mL of verbenone was instilled in the right conjunctival sacs of six rabbits, with the left eye of each rabbit serving as an untreated control. The eyes were rinsed after 24 hours. Observations for eye damage were made at 1 hour as well as 1, 2, 3, 4, and 7 days. Mild iritis (Draize score = 1) was observed in two of six animals. This effect cleared by day 7 after exposure. Mild to severe conjunctivitis and discharge was observed in all treated eyes. This effect also cleared by day 7. No corneal opacity or other signs of corneal damage were observed in any animals.

3.1.7. Systemic Toxic Effects from Dermal Exposure. In general, most occupational exposure scenarios and many of the exposure scenarios for the general public involve the dermal route of

exposure. Given the proposed use of verbenone, most scenarios involving dermal exposure to verbenone are irrelevant (section 3.2). Nonetheless, the potential for dermal absorption is important to the exposure scenarios that are plausible.

The available data on verbenone suggest that it is less toxic by dermal administration than by oral administration. As summarized in Appendix 1, oral doses as low as 1500 mg/kg caused mortality in female rats, whereas dermal doses of 2000 mg/kg caused no mortality in male or female rabbits (Deenihan 1987a,b,c).

There is no available information regarding the dermal absorption kinetics of verbenone. For exposure scenarios like an accidental spill, which involve deposition of the compound on the skin surface, dermal absorption rates (proportion of the deposited dose per unit time) are used in the exposure assessment. Using the methods discussed in Durkin et al. (1998), the estimated first-order dermal absorption coefficient is estimated at 0.014 $hour^{-1}$ with 95% confidence intervals of 0.0042 to 0.05 $hour^{-1}$. The calculations for these estimates are summarized in worksheet 10 and detailed in worksheet 08.

As discussed in Durkin et al. (1995), scenarios involving immersion or prolonged contact with solutions containing a compound use Fick's first law and require an estimate of the permeability coefficient, K_p, expressed in cm/hour rather than a first order dermal absorption rate. Using the method recommended by U.S. EPA (1992), the estimated dermal permeability coefficient (zero-order) for verbenone is 0.0075 cm/hour with a 95% confidence interval of 0.005 to 0.011 cm/hour. These estimates are used in all the exposure assessments based on Fick's first law. The calculations for these estimates are summarized in worksheet 10 and detailed in worksheet 09.

3.1.8. Inhalation Exposure. There is no information regarding the toxicity of verbenone by inhalation exposure in the published literature or in the U.S. EPA FIFRA/CBI files.

3.1.9. Impurities, Adjuvants, and Metabolites.

3.1.9.1. Impurities – Very few chemical reactions lead to only a single compound. Thus, most technical grade pesticides as well as other technical grade chemicals contain impurities that arise during the process of synthesis. Verbenone contains impurities, and the identity of the known impurities (e.g., LaFontaine and Williamson 1988a,b,c;d; LaFontaine and Williamson 1996; Phero Tech Inc. 1993c) were disclosed to the U.S. EPA and reviewed as part of this risk assessment. This information, however, is considered to be proprietary and cannot be disclosed in this risk assessment. The current product label of the pouch formulation specifies that the formulation contains 4.46 g of verbenone and 0.14 g of related compounds (Phero Tech Inc. 2000). Information regarding the potential toxicity of the impurities in verbenone was not encountered in the available literature.

Since the available literature regarding the toxicity of verbenone itself is extremely limited, the lack of information regarding the toxicity of the impurities in verbenone has relatively little impact on this risk assessment. Furthermore, the available toxicity studies on verbenone involve the use

of technical grade verbenone, which means that they at least partially encompass the toxicity of the impurities..

3.1.9.2. Metabolites – There is no information regarding the metabolism of verbenone in mammals in the published literature or in the U.S. EPA FIFRA/CBI files.

3.1.9.3. Adjuvants – As noted in section 2, verbenone formulations contain Ethanox 330. Ethanox 330 is an antioxidant provided by Ethyl Chemicals Group [1,3,5-trimethyl-2,4,6-tris(3,5-tert-butyl-4-hydroxybenzyl)benzene CAS 1709-70-2]. Ethanox 330 compound has an acute oral LD_{50} > 15,000 mg/kg and is allowed by the FDA in polymers for food contact applications. In a 90-day feeding study, a dietary concentration of 3.16% did not cause adverse effects in rats (Deenihan 1987a, p. 38 of 44).

3.1.10. Toxicological Interactions. Pintabona et al. (1995) studied the effect of (-)verbenone on the pharmacokinetics of two antibiotics: erythromycin and ampicillin, reporting that verbenone increases the concentration of both antibiotics in the lung but has no effect on the concentration of the antibiotics in the liver. No other information regarding the toxic joint action of verbenone with other compounds was found in the published literature or in the U.S. EPA FIFRA/CBI files.

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview. Many chemicals used by the Forest Service are applied by a relatively standard set of methods, including aerial broadcast, ground broadcast/mechanical, or backpack. For those methods of pesticide or herbicide application, a relatively consistent set of exposure scenarios was developed to assess exposure for workers and the general public (Rubin et al. 1998). Most of those exposure scenarios and approaches to exposure assessment are not relevant to the application method proposed for verbenone.

Under typical conditions of placing the verbenone pouch, workers should not be exposed to substantial levels of verbenone. The verbenone is encased in a sponge matrix that releases only very small quantities of verbenone per unit time. Without violating the proprietary nature of the packaging material, it can be stated that the entire verbenone sponge is encased in material that is impermeable to verbenone. Were this not true, the pouch formulation would have an unacceptably short shelf life.

There are no studies regarding worker exposure to verbenone. Although the default methods for estimating worker exposure are likely to lead to gross overestimates of absorbed dose, these default methods are applied for worker exposure in the absence of any other method to estimate exposure. Using these methods, routine applications of verbenone are estimated to lead to absorbed doses of 0.1 (0.01-0.3) mg/kg/day.

Two accidental exposure scenarios are also considered: inadvertent dermal contact during placement of the verbenone pouch by workers and imprudent handling of the pouch by a child. The worker scenario postulates that the pouch is ruptured in some way during its removal from

the packaging material or during its placement on a tree. Based on the assumption that both hands are contaminated with verbenone for 1 minute, the estimated absorbed dose is 1.5 (0.97-2.1) mg/kg bw. In a worst case and perhaps highly implausible exposure scenario, in which the worker does not clean the contaminated hands for 1 hour, exposure will not exceed 63.7 mg/kg. In this case, the dose estimate is limited by the amount of verbenone contained in a single pouch.

Like workers, members of the general public usually would not be exposed to substantial levels of verbenone. Nonetheless, as a worst case scenario, it is assumed that a child could encounter a pouch that was accidently dropped on to the ground or removed in some way from a tree. In this scenario, both dermal and oral exposure could occur through imprudent handling of the verbenone pouch. The estimated absorbed doses for the scenario range from 35 to 350 mg/kg bw.

3.2.2. Workers. Many chemicals used by the Forest Service are applied by a relatively standard set of methods, including aerial broadcast, ground broadcast/mechanical, or backpack. For those methods of pesticide or herbicide application, a relatively consistent set of exposure scenarios was developed to assess exposure for workers and the general public (Rubin et al. 1998). Most of those exposure scenarios and approaches to exposure assessment are not relevant to the application method proposed for verbenone. Any number of accidental exposure scenarios could be developed. For this risk assessment, one extremely conservative accidental exposure scenario is developed that involves the contamination of gloves during the application process.

3.2.2.1. General Exposures **--** No studies regarding worker exposure to verbenone or worker exposure to other chemicals in pouch formulations were found in the available literature. As discussed in section 2.2, the verbenone in the pouch formulations is in a sponge matrix, which is in turn encased in a pouch. While a pouch will release small quantities of verbenone once any protective packing material is removed, the risk of toxicologically significant exposure to verbenone appears to be unlikely under typical conditions of exposure (i.e., nailing the pouch to a tree).

As discussed in section 2.2, a worker could handle approximately 400-2500 verbenone pouches per day. Since each pouch contains 4.65 g (0.00465 kg) of verbenone, a worker could 'handle' about 1.86 to11.6 kg verbenone per day:

$$0.00465 \text{ kg verbenone/pouch} \times 400 \text{ to } 2500 \text{ pouches/day} = 1.86 \text{ to } 11.625 \text{ kg/day}$$

or about 4.1 to 25.6 lbs/day.

Methods are available for estimating absorbed doses in workers based on the amount of chemical handled per day (Rubin et al. 1998). The methods are based on data involving various forms of broadcast or directed spraying in which the mode of exposure involves direct contact of a chemical solution with the skin. For example, the exposure rate for workers involved in directed

foliar applications is 0.003 (0.0003-0.01) (mg agent/kg bw) ÷ (lbs agent handled per day) (Rubin et al. 1998, Table 5). These methods are not suitable for estimating exposure to pouch formulations because the verbenone is contained in a sponge matrix and direct contact of the chemical with the skin seems implausible. Thus, using the relationships developed by Rubin et al. (1998) to estimate verbenone exposure is likely to result in an overestimate of exposure by a very substantial margin. Notwithstanding these reservations, worker exposure could be estimated at 0.08 (0.008-0.3) mg/kg/day:

0.003 (0.0003-0.01) (mg agent/kg bw) ÷ (lbs agent handled per day) × 25.6 lbs/day

using the upper range of the amount of verbenone handled per day and rounding to one significant decimal.

3.2.2.2. Accidental Exposures – A worker could accidentally rupture a pouch containing 4.46 g or 4460 mg verbenone and contaminate the surface of the hands with verbenone. For this risk assessment, a more conservative exposure scenario is used. It is assumed that the worker accidentally ruptures a pouch and contaminates the inside of protective gloves with verbenone. This scenario is extremely conservative and perhaps implausible. In general, protective gloves will prevent dermal absorption. Nonetheless, this scenario is based on the assumption that the inside of the protective gloves is contaminated and that the gloves serve as a poultice, preventing the evaporation of verbenone and keeping the verbenone in contact with the exposed skin.

Under these conditions, the absorbed dose may be calculated assuming zero-order absorption. Any duration of exposure could be used. For this risk assessment, two durations are calculated: 1 minute and 1 hour. An exposure duration of 1 minute is based on the reasonable assumption that the worker promptly terminates exposure by removing the gloves and cleaning the hands. The 1-hour exposure duration assumes that the worker does not behave prudently.

The calculations for these exposure scenarios are detailed in worksheet 11. For a 1-minute exposure period, the absorbed dose is 1.5 (0.97-2.1) mg/kg. If a body weight of 70 kg is assumed for the worker, as specified in worksheet 02, the total absorbed dose is 105 (67.9-147) mg [70 kg × 1.5 (0.97-2.1) mg/kg]. Since each pouch contains 4460 mg of verbenone, the value for dermal absorption is equivalent to approximately 2.4%(1.5-3.3%) of the verbenone in a single pouch [100 × 105 (67.9-147) mg ÷ 4460 mg].

As also detailed in worksheet 11, the dose absorbed over a 1-hour period would be 87 (58-130 mg/kg). This corresponds to a total absorbed dose of 6090 (4060-9100) mg [70 kg × 87 (58-130 mg/kg)]. The lower range of this estimate, however, approaches the amount of verbenone present in a single pouch, and the central and upper estimates exceed the amount available in a single pouch. In other words, under the assumption of zero-order absorption with a duration period of 1 hour, the gloves would have to be contaminated with essentially all of the verbenone that is contained in a single pouch to approach even the lower limit of exposure. Thus, for this

aspect of the exposure assessment, the maximum possible absorbed dose is taken as 63.7 mg/kg, the total amount of verbenone in a singe pouch (4460 mg) divided by the body weight of 70 kg.

3.2.3. General Public. As is the case for workers, the general public should not be exposed to high levels of verbenone. Nevertheless, accidental exposure scenarios can be developed to reflect essentially arbitrary and very conservative estimates.

The scenario considered quantitatively in this risk assessment involves a small child coming into contact with a verbenone pouch dropped inadvertently during application. Variations of this scenario can be developed in which the child then effectively absorbs all or part of the verbenone either by ingestion or dermal exposure. If a body weight of 13 kg is used for a 2- to 3-year-old child (worksheet 03), the total dose would be approximately 343 mg/kg (i.e., 4460 mg/13 kg bw). This dose represents an upper limit.

More plausible estimates of the amount that might effectively be absorbed or consumed cannot be determined analytically. For this exposure assessment, a range from 35 to 350 mg/kg bw, with a central estimate of 100 mg/kg, is used. The lower range is based on the arbitrary assumption that about 10% of the available verbenone is consumed or otherwise absorbed (35 mg/kg bw), and the central estimate is based on the approximate geometric mean of the range (i.e., $(35\times350)^{0.5} \approx 112$ mg/kg).

More conservative scenarios could be developed; however, in the more conservative scenarios, the child must either come into contact with numerous pouches that were discarded or otherwise misplaced or actively seek out and consume pouches. Although these events are not unimaginable, they are not plausible enough to be considered quantitatively.

3.2.4. Inhalation Exposures. As discussed in section 2.1, verbenone pouches are designed to release about 115 to 230 mg/day over a 20 to 40 day period under normal field conditions (LaFontaine and Williamson 1996). Thus, inhalation exposures will probably be the most common form of exposure for both workers and the general public.

Monitoring studies of verbenone in air are not available. As a very crude approximation, a concentration in air can be calculated based on the daily release rate of verbenone from the pouch, an approximate application, and the assumption that the verbenone remains in a fixed volume of air. For this assessment, it is assumed that verbenone is applied at application rates of about 0.8 to 1.2 kg/ha, as detailed in Section 2.2. In addition, it is assumed that all of the verbenone is released from the pouch in a 20 day period. Thus, the daily release over a hectare area would be about 40,000 to 60,000 mg:

0.8 to 1.2 kg/ha ÷ 20 days = 0.04 to 0.06 kg/day = 40,000 to 60,000 mg/day per hectare

As also detailed in Section 2.2, verbenone pouches are applied to the trees at a height of 15 feet above ground. For this assessment, it will be assumed that the verbenone is distributed between

the ground surface and 15 feet above the application site (i.e., 30 feet or 9.1 m above the ground). Thus, the concentration in air may be estimated at about 0.4 to 0.7 mg/m^3:

$$40{,}000 \text{ to } 60{,}000 \text{ mg/day} \div (10{,}000 \text{ m}^2 \times 9.1 \text{ m}) = 0.44 \text{ to } 0.66 \text{ mg/m}^3.$$

where 10,000 m^2 is equal to one hectare.

Taking a reference volume of 22,800 L or 22.8 m^3/day for the volume of air breathed each day by a 70-kg reference man (ICRP, 1975, p. 346) and assuming that all of the inhaled verbenone is retained, the absorbed dose is equivalent to about 0.1 to 0.2 mg/kg/day:

$$0.4\text{-}0.7 \text{ mg/m}^3 \times 22.8 \text{ m}^3\text{/day} \div 70 \text{ kg} = 0.13\text{-}0.228 \text{ mg/kg}.$$

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview. Except for standard acute toxicity studies and some reports from the Italian literature on the anti-inflammatory activity of verbenone, there is no information on dose-response relationships for verbenone in humans or experimental mammals. Doses as low as 1000 mg/kg might be expected to cause death in rats. And 60 mg/kg is the reported NOAEL for anti-inflammatory activity in rats after exposure to verbenone.

3.3.2. Existing Guidelines. Neither the U.S. EPA nor the Agency for Toxic Substances and Disease Registry (ATSDR) have derived or recommended levels of acceptable exposure for this compound (i.e., RfDs or MRLs). Verbenone is not listed on the INTERNET sites of any of the organizations responsible for setting occupational exposure recommendations, criteria or standards (i.e., OSHA, NIOSH, or ACGIH). Furthermore, publications from these agencies and organizations regarding occupational exposure to verbenone were not encountered in the literature search, which included databases covering the Federal Register.

3.3.3. Dose/Severity Relationships. The information available on verbenone is not sufficient to derive any estimate of acceptable exposure to this compound for chronic scenarios.

As summarized in section 3.1.2 and detailed in Appendix 1, the lowest reported acute oral LD_{50} for verbenone is 1800 mg/kg with a 95% confidence interval of 1000-3240 mg/kg. This value is from a gavage study in rats (Deenihan 1987a,b,c). While various methods can be used to extrapolate such information to potentially lethal exposures in humans, the data on verbenone are insufficient to support such extrapolation. The study by Deenihan (1987a,b,c) also reports that dermal doses of 2000 mg/kg were not lethal to male or female rats.

The study by Chiariello et al. (1986c), which involved oral dosing of rats, reports an acute oral NOAEL for anti-inflammatory activity of 60 mg/kg with an effect level of 120 mg/kg. This study is from the Italian literature, and a translation of the study is not available. Nonetheless, this study represents what is probably the most relevant information for assessing the consequences of acute exposure to verbenone.

3.4. RISK CHARACTERIZATION

3.4.1. Overview. Risks are typically characterized quantitatively as a hazard quotient, which is an estimated level of exposure divided by some expression of an acceptable level of exposure. For verbenone, such quantitative characterizations of risk are not justified because of the very limited data available on the toxicity of this compound as well as the uncertainties in the estimates of the exposure levels.

Notwithstanding these limitations, there is no indication that toxicologically significant exposures to verbenone are plausible under typical conditions of use for either workers or members of general public. Based on the very limited information that is available on verbenone, it appears that implausibly high estimates of worker exposure to verbenone are below the most sensitive endpoint that is known (i.e., a NOAEL of 60 mg/kg for acute anti-inflammatory activity in rats). Accidental exposure for workers could result in absorbed doses that might reach or slightly exceed the NOAEL for rats exposed to verbenone. Very extreme accidental exposure scenarios for the general public—specifically a small child ingesting all of the verbenone in a single verbenone pouch—result in estimates of absorbed dose that approach the LD_{50} in rats.

The available information does not permit an assessment of the potential consequences of longer-term exposure to verbenone. Verbenone is a naturally occurring substance; however, this fact has no impact on the characterization of risk. Although many naturally occurring substances are benign, even essential, others can be extremely harmful.

This risk assessment for potential human health effects is, therefore, dominated by uncertainty (i.e., a lack of knowledge). Based on what is known about the toxicity of verbenone, the potential risks that the use of verbenone in Forest Service programs might pose to workers or members of the general public cannot be characterized well.

3.4.2. Workers. Because reliable estimates of worker exposure cannot be made and because of the very limited toxicity data on verbenone, a quantitative risk characterization is not justified. Nonetheless, the limited information that is available does not suggest a qualitative risk.

As shown in section 3.2.2.1, worker exposure, based on the amount of verbenone that could be handled by a worker in a single day, is estimated to be 0.08 (0.008-0.3) mg/kg/day, provided the compound is applied like herbicides are (i.e., directed or broadcast ground applications). Because of the actual way that verbenone is applied, the estimated dose is likely to be much greater than might reasonably be expected to occur. Nonetheless, the range of estimated dose levels is from 200 to 7500 time less than the acute NOAEL of 60 mg/kg for anti-inflammatory activity in rats. Similarly, the range of estimated levels of exposure is from approximately 3000 to 125,000 times less than the lower limit of acute LD_{50} in rats (1000 mg/kg). Although these ratios cannot be interpreted in the same way as hazard quotients, they are not a basis for asserting that workers would be at risk.

Accidental exposure scenarios for workers (see section 3.2.2.2) lead to dose estimates ranging from 1.5 (0.97-2.1) mg/kg (1-minute exposure) to 63.7 mg/kg (1-hour exposure). The dose for the 1-hour exposure period somewhat exceeds the rat NOAEL of 60 mg/kg. As detailed in section 3.2.2.2 and Worksheet 11, the value of 63.7 mg/kg for the 1-hour exposure is based on the extremely conservative assumption that all of the verbenone in a single pouch is absorbed by the worker.

3.4.3. General Public. As with the risk characterization for workers, the available data on verbenone do not support a quantitative risk characterization for members of the general public. Nonetheless, members of the general public are not likely to be exposed to significant levels of verbenone, under normal conditions of use and exposure. In the case of accidental tampering with a verbenone pouch by a small child, the estimated absorbed dose is 100 mg/kg with a range of 35-350 mg/kg bw. This range is a factor of about 3-30 below the lower limit of acute LD_{50} in rats (1000 mg/kg), which might raise concern for the possibility that extreme exposures could result in fatal poisoning incidents.

3.4.4. Sensitive Subgroups. There is no information to support the identification of groups that might be at special risk to verbenone exposure.

3.4.5. Connected Actions. There is no information to support the identification of other actions or exposures to other compounds that might alter responses to verbenone. As indicated in section 3.1, verbenone may impact the pharmacokinetics of some antibiotics. This effect is likely to be quantitatively substantial only at high doses. Under anticipated conditions of application, such an effect seems implausible.

3.4.6. Cumulative Effects. As discussed in section 3.1, there is no information regarding the subchronic or chronic toxicity of verbenone. Consequently, the potential effects of repeated exposures to this compound cannot be assessed.

3.4.7. Inhalation Exposures. As discussed in section 3.2.4., inhalation exposure is likely for both workers and members of the general public. The available data, however, are not adequate to assess plausible levels of exposure and there are no data regarding the inhalation toxicity of verbenone to humans or experimental mammals. Based on an extremely crude approximation of exposure that ignores both wind dispersion and photo-degradation and assumes 100% absorption of inhaled verbenone, absorbed doses may be estimated as 0.1-0.2 mg/kg/day (see section 3.2.4). These doses, which are probably gross overestimates, are below the a NOAEL of 60 mg/kg for acute anti-inflammatory activity in rats by factors of 300 to 600. Again, the limitations in both the exposure assessment and dose-response assessment do not justify a quantitative characterization of risk. Nonetheless, these crude analyses do not raise substantial concern based on the information that is available.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview. Like the human health risk assessment, the ecological risk assessment is governed by the limitations of the toxicity data on verbenone. The acute toxicity of verbenone was determined in rats (as discussed in the human health risk assessment), bobwhite quail, three species of terrestrial invertebrates, two fish species, and one aquatic invertebrate. There are few additional data on verbenone that affect this risk assessment. As in the case of the human health risk assessment, the lack of information regarding the toxicity of verbenone is mitigated somewhat by the limited use that the Forest Service proposes for the compound.

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 mammalian toxicity of verbenone is not well characterized. Verbenone seems to cause anti-inflammatory effects in rats. Although this effect is biological, it is usually regarded as therapeutic rather than adverse. At least in rats, the only species on which data are available, the limited toxicity data are not sufficient to assess the effects of subchronic or chronic exposure to verbenone.

4.1.2.2. Birds– There is only one study regarding effects in birds after exposure to verbenone (Grimes and Jaber 1987a,b). Like the study regarding the toxicity of verbenone to experimental mammals (Deenihan 1987a,b,c), the study on birds was submitted to the U.S. EPA in support of the registration of verbenone. Since the study was submitted twice, it was apparently used to support two formulations of verbenone.

In the study by Grimes and Jaber (1987a,b), verbenone was administered to bobwhite quail (21-weeks old, weighing about 0.18 to 0.22 kg, in groups of five quail/sex/dose) by gavage at doses of 0 (vehicle control), 39, 65, 108, 180, or 300 mg/kg in corn oil. Body weights were measured on days 3, 7, and 14 after dosing. No mortality and no changes in behavior, body weight, or food consumption were noted. Thus, Grimes and Jaber (1987a,b) report the LD_{50} as >300 mg/kg. This is consistent with the mammalian data, which indicates LD_{50} values (with 95% confidence intervals) of 3400 (2833-4080) mg/kg for males rats and 1800 (1000-3240) mg/kg for female rats (Deenihan 1987a,b,c).

4.1.2.3. Terrestrial Invertebrates– The activity of verbenone in target species is relatively well documented. Verbenone is one of a series of naturally occurring semiochemicals—including á-pinene, frontalin, endo-brevicomin, trans- and cis-verbenol, ipsdienol, and ipsenol—which serve as pheromones (both attractant and disaggregant) in inter-and intra-species communication of several pest species (Ascoli-Christensen et al. 1993; Grosman et al. 1997; Miller et al. 1995; Paine and Hanlon 1991).

At least in the southern pine beetle, *Dendroctonus frontalis*, verbenone is found at much greater concentrations in males (about 3000-8000 ng/beetle) than in females (about 20-120 ng/beetle)

(Grosman et al. 1997, Table 1, p. 440). As indicated in section 2, verbenone can exist in either of two enantiomers and the relative efficacy of different enantiomer mixtures has been characterized in the southern pine beetle, *Dendroctonus frontalis*, in which different enantiomer mixtures had differing efficacy in males but not females (Salom et al. 1992). Efficacy in males but not females was observed also in the pales weevil, *Hylobius pales* (Salom et al. 1994). Conversely, verbenone is effective in the spruce bark beetle, *Ips paraconfusus*, in reducing the number of females but not males reaching an attractant source (McPheron et al. 1997).

The only information on the toxicity of verbenone to nontarget terrestrial insects comes from a study by Rice and Coats (1994) in which a series of monoterpenoids and related compounds were assayed for direct contact and vapor toxicity to house flies and a flour beetle as well as toxicity to southern corn rootworm larvae and house fly eggs. The results of this study are discussed further in the dose-response assessment for terrestrial invertebrates (section 4.3.2.3).

4.1.2.4. Terrestrial Plants (Macrophytes)– Studies regarding the toxicity of verbenone to terrestrial macrophytes are not available in literature. Verbenone is a natural constituent of some terrestrial macrophytes, specifically some species of pine. As in insects and microorganisms, verbenone is synthesized by plants from á-pinene via verbenol (Vanek et al. 1989, 1994). While this does not demonstrate that verbenone is non-toxic to plants, it suggests that adverse effects in terrestrial plant species is unlikely, given the types of applications anticipated in Forest Service programs. In addition, several field trials of various formulations of verbenone were conducted, and no adverse effects were observed in terrestrial plants. If the field trials had resulted in substantial damage to terrestrial plants from the application of verbenone, it is likely that such effects would have been noted.

4.1.2.5. Terrestrial Microorganisms– Data regarding the toxicity of verbenone to terrestrial microorganisms are not available in the literature. Verbenone can be formed by some microorganisms in the metabolism of á-pinene (e.g., Van Dyk et al. 1998). This action was documented in yeasts associated with pine bark beetles (*Dendroctonus ponderosae*) (Kostyk et al. 1993) and spruce bark beetles (*Ips typographus*) (Leufven et al. 1984). Although this effect does not demonstrate that verbenone will not be toxic to all species of terrestrial microorganisms, it lends some credence to the presumption that verbenone is not likely to have an adverse effect on terrestrial microorganisms, given the way in which verbenone will be used in Forest Service programs.

4.1.3. Aquatic Organisms.

4.1.3.1. Fish– Information on the toxicity of verbenone to fish is summarized in Appendix 2. Verbenone was tested in acute bioassays of bluegill sunfish (Surprenant 1988e,f) and rainbow trout (Surprenant 1988a,e). Like the acute study using bobwhite quail, both of the fish studies were submitted to the U.S. EPA in support of the registration of verbenone, and, since both studies were submitted twice, it appears they were used to support the registration of two formulations.

Based on nominal concentrations, the 96-hour LC_{50} value (with a 95% confidence interval) for bluegills is 210 (130-360) mg/L (Surprenant 1988e,f). The corresponding value for trout is 130 (100-160) mg/L (Surprenant 1988a,e). In both of these bioassays, as well as in the daphnid bioassay summarized in section 4.1.3.3, a film of undissolved material was observed on the surface of the test water at all concentrations. As summarized in Table 2-1, verbenone is practically insoluble in water. Thus, it is very likely that the nominal water concentrations reported in these studies were greater than the actual concentration of the test material in the water.

4.1.3.2. Amphibians– Information regarding the toxicity of verbenone to amphibians is not available in the literature.

4.1.3.3. Aquatic Invertebrates– Verbenone was tested in an acute bioassay using *Daphnia magna* (Surprenant 1988b,d). The reported 24- and 48-hour LC_{50} values (with a 95% confidence interval) are 340 (300-390) mg/L and 200 (130-360) mg/L, respectively. As observed in the fish bioassays, a film of undissolved material was observed on the surface of the test water at all concentrations. There were, however, no substantial changes in the concentration of dissolved oxygen in the test water.

4.1.3.4. Aquatic Plants– Data regarding the toxicity of verbenone to aquatic plants is not available in the literature.

4.1.3.5. Other Aquatic Microorganisms– Data regarding the toxicity of verbenone to aquatic microorganisms is not available in the literature.

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview. As with the human health risk assessment, there is a set of relatively consistent exposure scenarios typically developed for terrestrial and aquatic animals and plants. These scenarios are developed for chemicals applied by aerial broadcast, ground broadcast/mechanical, or backpack spray. Because of the limited manner in which verbenone will be used and applied by the Forest Service, however, most of the typical exposure scenarios are not applicable to this risk assessment.

The only exposure scenarios developed for quantitative use in the risk characterization involve the animal consumption of verbenone from a pouch formulation. This scenario is plausible, given that the placement of verbenone pouches is likely to make them available to terrestrial species.

Quantitative exposure scenarios for aquatic species are not plausible. Nonetheless, the extremely low levels of verbenone that might be found in water are taken into consideration.

4.2.2. Consumption by Terrestrial Animals. Because verbenone will be placed on trees, some terrestrial organisms could tamper with the pouch and consume its contents. Several exposure

scenarios could be developed for various species of terrestrial animals; however, the usefulness of those assessments is limited by the available toxicity data (section 4.3).

The consumption of the verbenone in a pouch formulation most closely parallels gavage administration. Because there are data regarding effects in rats and quail after acute exposure to verbenone via gavage administration, exposure scenarios involving the consumption of verbenone in a pouch by rats and quail can be developed directly from the available data. Extrapolation of exposure to other species of birds or mammals can be made by calculating dose rates based on known differences in body weight (U.S. EPA 1993).

A major uncertainty, of course, is in the amount of verbenone that an animal might ingest. As indicated in section 2, each verbenone pouch weighs 6.28 g and contains 4.65 g of verbenone. For small animals, it is unreasonable to assume that they would consume the entire pouch. For example, small rodents typically consume food amounts equivalent to about 15% of their body weight per day. For a 20 g animal, this amount is equivalent to about 3 g or 3000 mg. Thus, it seems unreasonable to assume that animals of that size would consume all of the material within a single pouch.

For this risk assessment, it is assumed that the animal consumes an amount of verbenone that is equal to 20% of the amount of food that it would normally consume in a day up to a maximum of 4650 mg, the total amount of verbenone in a single pouch. A summary of the ingested doses for various species of birds and mammals is provided in Table 4-1. All of these scenarios are based on the general assumption that the animal encounters a recently placed pouch containing 4650 mg of verbenone. The potential impact of more extreme exposures is discussed in the risk characterization (section 4.4).

4.2.3. Vapor Exposures. As discussed in section 3.2.4 of the human health risk assessment, the most plausible route of exposure is from the release of verbenone from pouches into the surrounding air. Based on a very crude set of exposure assumptions, concentrations of verbenone in air are estimated to range from 0.1 to 0.2 mg/m^3. Because these estimates do not consider either dispersion by air currents or photo-degradation, they are likely to overestimate exposure substantially.

4.2.4. Aquatic Organisms. Given the method of verbenone application proposed by the Forest Service, there is no reason to assume that aquatic organisms will be exposed to significant levels of verbenone. Although it is possible to construct numerous accidental exposure scenarios involving relatively small amounts of verbenone (i.e., a pouch dropped into a pond), generating such scenarios would lead to trivial levels of exposure. For example, a one-quarter acre pond has a surface area of about 1000 m^2. If the pond has an average depth of 1 m, it will contain 1000 m^3, which is equivalent to 1,000,000 L of water (i.e., 1 m^3= 100 cm ×100 cm × 100 cm = 1,000,000 cm^3 = 1,000,000 mL = 1,000 L). If a pouch containing 4650 mg of verbenone were ruptured and released into the water, the concentration with instantaneous dilution would be 0.00465 mg/L [4,650 mg/1,000,000L] or 4.65 µg/L.

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Overview. Dose-response relationships for acute toxic effects can be characterized for rats, quail, three species of nontarget insects, and three aquatic organisms: bluegill sunfish, trout, and daphnids. As discussed in the human health risk assessment, the one available rat study reports mortality at doses as low as 1500 mg/kg after gavage administration. An acute study on anti-inflammatory activity reports a NOAEL of 60 mg/kg and an effect level of 120 mg/kg. The one available avian study (bobwhite quail) reports a NOAEL of 300 mg/kg/day based on the absence of overt signs of toxicity and changes in body weight. In aquatic species, the lowest reported 96-hour LC_{50} value is 130 (100-160) mg/L (trout). Given the proposed use of verbenone and the improbability of ambient water becoming contaminated with verbenone, these data are adequate for characterizing risk.

In the one publication regarding the toxicity of verbenone to nontarget terrestrial insects, mortality occurred after direct contact (176 µg/fly) or after vapor exposure (7-22 $\mu g/cm^3$). Moreover, soil concentrations of approximately 50 ppm caused mortality in about 50% of southern corn rootworm larvae, and liquid media concentrations of 833 ppm caused a 65% inhibition of housefly egg hatch.

Data regarding the toxicity of verbenone to terrestrial or aquatic plants or microorganisms are not available in the literature.

4.3.2. Toxicity to Terrestrial Organisms.

4.3.2.1. Mammals– Although the mammalian toxicity of verbenone is not well characterized, the available data regarding gavage administration of verbenone (Deenihan 1987a,b,c) are highly relevant to an assessment of the consequences of ingesting liquid verbenone from a pouch formulation. As detailed in Appendix 1 and discussed in section 3.3.3, gavage doses as low as 1500 mg/kg, the lowest dose tested in the Deenihan (1987a,b,c) study, resulted in the death of two of five treated female rats. A dose of 2500 mg/kg bw caused death in one of five male rats. Conversely, gavage doses of 60 and 120 mg/kg caused no deaths in rats, although the higher dose was associated with anti-inflammatory activity (Chiariello et al. 1986c). Thus, as in the human health risk assessment, 60 mg/kg is regarded as a NOAEL and doses approaching 1000 mg/kg are treated as dose levels that could be fatal in some animals.

4.3.2.2. Birds– Data regarding the toxicity of verbenone in bobwhite quail after exposure to the compound by gavage are provided in the study by Grimes and Jaber (1987a,b). In the study, no effects were observed in birds after single gavage doses of up to 300 mg/kg. As with mammalian toxicity studies, the available data on birds are inadequate to support a quantitative species to species extrapolation. This uncertainty is discussed further in the risk characterization (section 4.4).

4.3.2.3. Terrestrial Invertebrates– Although acute contact bioassays in bees are typically required by U.S. EPA for the registration of pesticides, this requirement appears to have been waived for verbenone. Recently, however, Rice and Coats (1994) published the results of a series

of bioassays in the housefly, red flour beetle, and southern corn rootworm. Data from this study are summarized in Table 4-2.

4.3.2.4. Terrestrial Plants (Macrophytes) and Microorganisms– There are no dose-response data regarding the toxicity of verbenone to terrestrial plants or microorganisms. As discussed in section 4.1, there is no basis for suggesting that verbenone would be toxic to either macrophytes or microorganisms under the conditions of use proposed by the Forest Service.

4.3.3. Aquatic Organisms.

4.3.3.1. Animals– As summarized in Appendix 2, the toxicity of verbenone to two species of fish, bluegills and trout, as well as one aquatic invertebrate, *Daphnia magna*, was assayed. Although the differences among species are not statistically significant, the lower range of the lowest LC_{50} is 100 mg/L. Given the improbability of significant exposure for aquatic species, this value is sufficient for the characterization of risk.

4.3.3.2. Aquatic Plants and Microorganisms– Data regarding the toxicity of verbenone to aquatic plants or microorganisms are not available in the literature.

4.4. RISK CHARACTERIZATION

4.4.1. Overview. As is true in the human health risk assessment, the overriding factor in the ecological risk assessment is the paucity of information available on the toxicity of verbenone. There are no data in the literature regarding the chronic toxicity or potential reproductive effects of verbenone, and the acute toxicity of verbenone was assayed in only a few species. The risk characterization is, therefore, limited. There is no evidence to suggest that exposure to verbenone will have a substantial impact on any species populations; nevertheless, exposure to toxic levels may affect individual organisms under conditions of extreme exposure.

Given the limited use of verbenone proposed by the Forest Service, two exposure scenarios are plausible: tampering with a pouch and exposure to very low levels of verbenone in the air. If an animal were to tamper with a verbenone pouch, the amount of verbenone that might be consumed or otherwise absorbed could range from negligible to the total amount of verbenone in the pouch, which is about 4650 mg. The consequences of such an event will vary depending on the size of the animal. Small to medium sized mammals ranging in body weight from 20 to 3000 g might be exposed to lethal doses of verbenone. Larger mammals are less likely to be exposed to a lethal dose—provided that they tampered with only one verbenone pouch—but could be exposed to levels that are above the threshold for anti-inflammatory activity. It is unclear whether such exposures would cause adverse effects. If birds are as sensitive as mammals to verbenone exposure, a number of birds might also be exposed to lethal doses of verbenone, were they to tamper with the verbenone pouch.

The probability of any wildlife species consuming lethal amounts of verbenone cannot be assessed, based on the available data. The verbenone in pouches clearly would be available. Nonetheless, field studies were conducted on the efficacy of verbenone. If wildlife species commonly sought

and consumed verbenone, it is likely that the event would be reported in the field studies. Thus, despite the potential risk to an individual animal, it seems unlikely that the consumption of verbenone by wildlife will have a detectable or substantial impact on the population of any species.

Many species will be exposed to low levels of verbenone in air because of the proposed way in which the compound will be used by the Forest Service. For mammals and birds, there are no useful data for assessing the consequences of such exposure. Furthermore, there are no data regarding ambient air concentrations of verbenone, either from natural sources or from the application of verbenone in the field. Consequently, it is not possible to characterize risk quantitatively. Nonetheless, a very crude comparison made in the human health risk assessment of possible verbenone concentrations in air to estimates of verbenone that might be absorbed suggests that toxicologically significant levels of exposure are implausible. This conclusion is reinforced by data on insects, which suggest that exposure levels are likely to be 35,000 to 70,000 times less than lethal concentrations.

It is not plausible that aquatic species will be exposed to substantial levels of verbenone. Based on the acute toxicity data, there is no reason to believe that even extreme accidental exposure would have any impact on aquatic species.

4.4.2. Terrestrial Organisms.

4.4.2.1. Mammals– As discussed in section 4.2.2, the most plausible exposure scenario for terrestrial mammals involves tampering with the pouch formulation and consuming some of the enclosed verbenone. The amount of verbenone that would be consumed could range from 0 to 4650 mg (the amount of verbenone in an individual pouch). For small animals, it is unreasonable to assume that the entire pouch would be consumed. For the quantitative risk characterization, the assumption is made that each animal will consume an amount of verbenone that is equal to 20% of the amount of food that the animal would normally consume in a single day up to a maximum of 4650 mg. In assessing the potential consequences of these exposure scenarios in mammals, a dose of 60 mg/kg is used as a NOAEL for anti-inflammatory activity. This is the only NOAEL available for acute exposure in mammals. A dose of 1000 mg/kg is used as the approximate lethal dose. This value is somewhat less than the lower limit of the LD_{50} values in mammals. There is no estimate of a lethal dose in birds. The only available information is that doses of up to 300 mg/kg are apparent NOAELs in birds based on crude measures of toxicity (i.e., survival, body weight, and gross signs of toxicity).

Based on the exposure estimates summarized in Table 4-1, it is apparent that several small to medium sized mammals ranging in body weight from 20 to 3000 g could be exposed to lethal doses of verbenone. Larger mammals are much less likely to be exposed to a lethal dose—provided that they tampered with only one verbenone pouch—but could be exposed to levels greater than the threshold for anti-inflammatory activity. It is unclear whether such exposures would cause adverse effects.

This interpretation is predicated on the assumption of equal sensitivity among species to the toxic effects of verbenone. Wildlife species are not commonly used as experimental animals; consequently, direct data on the toxicity to species of concern are seldom available. Nonetheless, for well-studied compounds, there might be information about various experimental mammals, which would allow for an assessment regarding sensitivity differences among species. There are no such data for verbenone.

This lack of information results in uncertainty in the risk characterization. Notwithstanding this uncertainty, the limited nature of plausible exposures should be appreciated. There is no evidence to suggest that adverse effects are likely to result from exposure to verbenone, unless an animal actually eats the material in the pouch formulation. The likelihood that numerous individuals of any species will consume the material in the verbenone pouch cannot be determined. Several efficacy studies were conducted on verbenone and no incidents of wildlife tampering with the verbenone formulations were reported. Thus, in practical terms, the potential risk to wildlife species may be negligible.

4.4.2.2. Birds– The available toxicity data on birds do not permit a clear assessment of the consequences of exposure to verbenone. All that is known is that 300 mg/kg is not lethal to bobwhite quail and doses of 1000 mg/kg may be lethal to rats. These data need not be interpreted as evidence that birds are more or less sensitive than mammals to verbenone exposure. If birds are as sensitive as mammals to verbenone, numerous birds might be exposed to lethal doses of the compound as a result of tampering with the verbenone pouch, the likelihood of which is not known.

4.4.2.3. Terrestrial Invertebrates– Verbenone will affect the behavior of numerous pest species. This, which is the intended effect of verbenone, is demonstrated in several efficacy studies identified in section 2. There is no information indicating whether verbenone might act as a pheromone in nontarget species. The only information on nontarget invertebrates is the series of bioassays conducted by Rice and Coats (1994), as summarized in Table 4-2.

The bioassays involving contact toxicity are not directly relevant to this risk assessment because direct contact with liquid verbenone is not a plausible scenario given the application method. It is, of course, conceivable that an insect or other invertebrate might come into direct contact with verbenone from a ruptured pouch and contaminate a large proportion of its body. In such a case, it is possible that the insect would be exposed to a lethal amount of verbenone. This type of isolated event involving a single animal, however, would have no measurable impact on the environment.

A more common and indeed inevitable exposure involves verbenone vapor. As discussed in section 4.2.3, an extremely conservative exposure assessment leads to estimated concentrations of verbenone in air ranging from 0.1 to 0.2 mg/m^3. As summarized in Table 4-2, the lower limit of the LD_{50} for the most sensitive species on which data are available is 7 $\mu g/cm^3$. This is equivalent to 7 mg/L [1000 cm^3 per liter] or 7,000 mg/m^3 [1000 L per m^3]. Thus, the estimated air

concentrations are factors of 35,000 to 70,000 below the lower limit of the LC_{50}. As with the risk characterization for mammals and birds, the limited nature of the available data do not warrant a quantitative expression of risk, but the information does suggest that exposure to verbenone is not likely to cause mortality in nontarget insects.

4.4.2.4. Other Terrestrial Species– There is insufficient information to assess the potential effects of verbenone on other species of terrestrial microorganisms or terrestrial plants.

4.4.3. Aquatic Organisms.

4.4.3.1. Fish and Aquatic Invertebrates–The acute toxicity of verbenone to fish and aquatic invertebrates has not been studied extensively; however, acute bioassays are available. Based on the exposure assessment presented for aquatic species (one pouch dropped into a small pond), the concentration of verbenone in water would be 4.65 µg/L. This amount is about 21,000 times less than the lower limit of the lowest reported 96-hour LC_{50} (i.e., 100 mg/L) (Surprenant 1988a,c in Appendix 2) [100 mg/L ÷ 0.00465 mg/L = 21,505]. In other words, about 21,000 pouches would have to be dropped into a small pond to result in a concentration likely to cause substantial mortality. Given how the Forest Service plans on using and applying these pouches, there does not appear to be plausible risk to aquatic animals.

4.4.3.2. Aquatic Plants– There are no data available on the toxicity of verbenone to aquatic plants. Nonetheless and as is the case with fish and aquatic invertebrates, the plausibility of toxicologically significant exposure seems remote. Thus, this particular data gap has no substantial impact on this risk assessment.

Table 4-1: Exposure for scenarios for the consumption of verbenone from a pouch formulation by mammals and birds.

Species	Body Weight (grams)	Food Consumed (grams) per day [a]	Verbenone consumed (mg) [b]	Reference for Body Weight	Estimated Dose[a] (mg/kg bw)
Mammals					
Short-tailed shrew	22	3.0	596	U.S. EPA 1993, p. 2-209	27,111
Rat, young albino	150	14	2,890	Deenihan 1987a,b,c [c]	19,264
Racoon, small	3000	170	4,650	U.S. EPA 1993, p. 2-233	1,550
Racoon, large	9000	418	4,650	U.S. EPA 1993, p. 2-233	517
Birds					
Robin	80	11	2,247	U.S. EPA 1993, p. 2-194, average for both sexes	28,082
Bobwhite quail	200	20	4,079	Grimes and Jaber 1987a,b	20,396
Mallard duck	1800	85	4,650	U.S. EPA 1993, p. 2-43, maximum weight	2,583
Herring Gull	1000	58	4,650	U.S. EPA 1993, p. 2-157	4,650
Bald eagle	5000	166	4,650	U.S. EPA 1993, p. 2-95, typical weight	930

[a] For mammals: $0.235\ BW^{0.822}$ (U.S. EPA, 1993, p. 3-6). For birds, $0.648\ BW^{0.651}$ (U.S. EPA, 1993, p. 3-4).
[b] 20% of normal food consumption up to a maximum of 4,650 mg, the amount of verbenone in a single pouch.
[c] Average of range of 150 to 250 g.

Table 4-2: Toxicity of verbenone to non-target insect species (data from Rice and Coats 1994).

Organism	Type of Assay	LC_{50}	Units
House fly (*Musca domestica*)	Topical, mortality at 24 hours.	176 (162-192)	µg/fly
	Vapor, mortality at 14 hours	7.7 (7.0 - 8.4)	$\mu g/cm^3$
	Ovicidal, exposure period of at least 4 days	833 [a]	ppm in solution
Red flour beetle (*Tribolium castaneum*)	Vapor, mortality at 24 hours	17.3 (13.5-22.1)	$\mu g/cm^3$
Southern corn rootworm, (*Diabrotica undecimpunctata*)	Larvicidal, mortality at 48 hours	46.5 (35.7-60.6)	ppm soil

[a] 65% inhibition of egg hatch

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Appendix 1: Toxicity of verbenone to experimental mammals.

Animal	Dose	Response	Reference
ORAL			
Rats, Sprague-Dawley, young male and female, 150 to 250 g, 5 rats per dose level per sex. Gavage in Mazola oil at a volume of 10 mg/kg bw.	Males: Doses of 0 (vehicle control), 2500, 3000, 3500, 4000, and 5000 mg/kg bw.	LD_{50} of 3400 mg/kg with 95% confidence interval of 2833 to 4080 mg/kg. At lowest dose (2500 mg/kg), mortality in 1/5 animals.	Deenihan 1987a,b,c MRID 40539902, 40551402, 42729601
	Females: Doses of 0 (vehicle control), 1500, 2000, 3000, and 5000 mg/kg bw.	LD_{50} of 1800 mg/kg with 95% confidence interval of 1000 to 3240 mg/kg. At lowest dose (1500 mg/kg), mortality in 2/5 animals.	Deenihan 1987a,b,c MRID 40539902, 40551402, 42729601

Notes on Above study: Females appear to be somewhat more sensitive than males although the differences are not statistically significant. All but one death occurred within 24 hours of dosing. Tremors and convulsions observed in test animals as well as weight loss and lethargy. Gross pathologic changes included mottled lungs, 'green material and gas in some of the animals' intestines, mottled liver, reddened adrenals. No mortality and no gross pathological changes in any of the control animals.

DERMAL			
Rabbits, New Zealand, 2 to 3 kg bw, 5 per sex.	Undiluted compound applied at a dose of 2000 mg/kg to abraded skin covering about 10% of body surface. Treated area covered for 24 hours after which time the residual test material was removed.	No mortality. All males and 3/5 females evidenced mild irritation in areas receiving test material (Draize scores of 1-2 for erythema and edema). After several days, sloughing of skin observed at test site in some animals. No abnormalities on necropsy.	Deenihan 1987a,b,c MRID 40539902, 40551402, 42729601

Appendix 1: Toxicity of verbenone to experimental mammals.

Animal	Dose	Response	Reference
Rabbits, New Zealand, 2 to 3 kg bw, n = 6. Sex not specified.	0.5 g applied to one intact area of skin and covered with one inch square gauze patches. Entire trunk of each animal wrapped for four fours, after which residual material was removed by washing.	Slight erythema in 2/6 animals. Mild edema in 1/6 animals.	Deenihan 1987a,b,c MRID 40539902, 40551402, 42729601

Appendix 1: Toxicity of verbenone to experimental mammals.

Animal	Dose	Response	Reference
OCULAR			
Rabbits, New Zealand, n = 6.	0.1 mL in right conjunctival sac. Eye rinsed after 24 hours. Eyes examined at 1 hour as well as 1, 2, 3, 4, and 7 days.	Moderate eye irritant. Mild iritis (Draize score = 1) in 2/6 animals that cleared by day 7. Mild to severe conjunctivitis and discharge in all animals that cleared by day 7. No corneal opacity.	Deenihan 1987a,b,c MRID 40539902, 40551402, 42729601

Appendix 2: Toxicity to fish and aquatic invertebrates. [a.i. technical unless otherwise specified]

Animal	Exposure	Response	Reference
Bluegill Sunfish (*Lepomis macrochirus*), mean length 40 mm, mean weight 0.69 g	Nominal concentrations of 0, 78, 130, 220, 360, and 600 mg/L for 96 hours at 20-21°C. Static exposure system. Aeration after 48 hours.	96-hour LC_{50}: 210 (130-360) mg/L. No mortality at two lower concentrations and 100% mortality at two higher concentrations. 60% mortality at 130 mg/L. All mortality occurred within first 24 hours. Film of undissolved material observed on surface of water at all concentrations. No concentration related differences in dissolved oxygen concentrations in water.	Surprenant 1988e,f MRID: 40539904, 42799102

Appendix 2: Toxicity to fish and aquatic invertebrates. [a.i. technical unless otherwise specified]

Animal	Exposure	Response	Reference
Rainbow trout (*Salmo gairdneri*), mean length 34 mm, mean weight 0.45 g	Nominal concentrations of 0, 39, 65, 110, 180, 300, and 500 mg/L for 96 hours at 20-21°C. Static exposure system.	96-hour LC_{50}: 130 (100-160) mg/L. No deaths at 39 or 65 mg/L. Some fish evidenced loss of equilibrium at 65 mg/L. Progressive mortality over time at concentrations of 110 and 180 mg/L. NOEL 39 mg/L. Film of undissolved material observed on surface of water at concentrations of 65 mg/L and greater. Slight decrease in dissolved oxygen concentrations in water related to concentration of verbenone at 48 hours and later.	Surprenant 1988a,c MRID: 40539906, 42799104

Appendix 2: Toxicity to fish and aquatic invertebrates. [a.i. technical unless otherwise specified]

Animal	Exposure	Response	Reference
Daphnia magna, < 24 hr old,	Nominal concentrations of 0, 130, 360, 600, and 1000 mg/L for 48 hours. Static exposures.	24-hour LC_{50}: 340 (300-390) mg/L. 48-hour LC_{50}: 200 (130-360) mg/L. Mortalities of 0, 0, 10, 50, 100, and 100% at 24 hours. Mortalities of 0, 0, 65, 100, 100, and 100% at 48 hours. Film of undissolved material observed on surface of water at all concentrations. No concentration related differences in dissolved oxygen concentrations in water.	Surprenant 1988b,d MRID: 40539905, 42799103

WORKSHEETS FOR
Verbenone

NOTE: Given the nature of the anticipated exposures to Verbenone, many of the standard worksheets used in Forest Service risk assessments are not used and have been omitted. In addition, many of the worksheets that are included have been substantially simplified.

Worksheet Table of Contents

GENERAL ASSUMPTIONS, VALUES, and MODELS

Worksheet 01: Constants and conversion factors used in calculations		
mg/lb	mg_lb	453,600
mL/gallon	ml_gal	3,785
lb/gallon to mg/mL	lbg_mgml	119.8
lb/acre to µg/cm^2	lbac_ugcm	11.21
lb/acre to mg/cm^2	lbac_mgcm	0.01121
gallons to liters	gal_lit	3.785

Worksheet 02: General Assumptions Used in Worker Exposure Assessments				
Parameter	Code	Value	Units	Reference
Body Weight (General)	BW	70	kg	ICRP (1975), p. 13
Surface area of hands	Hands	840	cm^2	U.S. EPA 1992

Worksheet 03: General Assumptions Used in Exposure Assessments for the General Public				
Description	ID	Value	Units	Reference
Body Weights				
Male, Adult	BWM	70	kg	ICRP (1975), p. 13.
Female, 45-55 years old, 50th percentile	BWF	64	kg	U.S. EPA, 1985, page 5, Table 2-2, rounded to nearest kilogram.
Child, male, 2-3 years old	BWC	13	kg	U.S. EPA, 1985, page 6, Table 2-3, rounded to nearest kilogram.

Worksheet 04: Estimate of first-order absorption rate (k_a in hours^{-1}) and 95% confidence intervals (from Durkin et al. 1998).			
Model parameters	ID	Value	
Coefficient for $k_{o/w}$	C_KOW	0.233255	
Coefficient for MW	C_MW	0.005657	
Model Constant	CONST	1.49615	
Number of data points	DP	29	
Degrees of Freedom (d.f.)	DF	26	
Critical value of $\boldsymbol{t}_{0.025}$ with 26 d.f.[1]	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
[1] 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 $\log_{10} k_a$

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

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 $\boldsymbol{a'X'X\,a}$ without using matrix arithmetic.

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: $\{a\_1 \times([a\_1 \times b\_1] + [a\_2 \times c\_1] + [a\_3 \times d\_1])\} +$
Term 2: $\{a\_2 \times([a\_1 \times b\_2] + [a\_2 \times c\_2] + [a\_3 \times d\_2])\} +$
Term 3: $\{a\_3 \times([a\_1 \times b\_3] + [a\_2 \times c\_3] + [a\_3 \times d\_3])\}.$

Worksheet 05: Estimate of dermal permeability (K_p in cm/hr) and 95% confidence intervals (data from U.S. EPA 1992).			
Model parameters	ID	Value	
Coefficient for $k_{o/w}$	C_KOW	0.706648	
Coefficient for MW	C_MW	0.006151	
Model Constant	CONST	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.[1]	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
[1] Mendenhall and Scheaffer, 1973, Appendix 3, Table 4, p. A31.			

NOTE: The data for this analysis is 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 EPA report, however, 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 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 greater number of decimal places to reduce rounding errors when calculating the confidence intervals. See notes to Worksheet 04 for details of calculating maximum likelihood estimates and confidence intervals.

CHEMICAL SPECIFIC VALUES

Worksheet 06: Anticipated Application and Dilution Rates for Verbenone				
Item	Code	Value	Units	Reference/Source
Typical application rate	Typ	Central estimates or range of application rates are not applicable to this risk assessment.		See Section 2.2 for details.
Lowest application rate	Low			
Highest application rate	Hi			
Note: Because verbenone is only applied to relatively small infested area, application rates calculated in the text are not comparable to those of compounds that may be broadcast over wide areas.				

Worksheet 07: Chemical specific values used for Verbenone in exposure assessment worksheets.				
Parameter	ID	Value	Units	Source/Reference
Molecular weight	MW	150.21	grams/mole	Budavari 1989
$K_{o/w}$	Kow	144.9	unitless	LaFontaine and Williamson 1988a
Estimate BCF	BCFC	20	kg fish/L	Calabrese and Baldwin, 1993 [a]
[a] Recommended equation for concentration in fish muscle (edible portion) is: $\log(BCF) = 0.54 \log (K_{o/w}) + 0.124$				

Worksheet 08: Calculation of first-order dermal absorption rate (k_a) for Verbenone.							
Parameters	Value	Units			Reference		
Molecular weight	150.21	g/mole					
$K_{o/w}$ at pH 7	144.9	unitless					
$\log_{10} K_{o/w}$	2.16						
Column vector ***a*** for calculating confidence intervals (see Worksheet 04 for definitions.)							
a_1	1						
a_2	150.21						
a_3	2.16						
Calculation of $\mathbf{a'} \cdot (\mathbf{X'X})^{-1} \cdot \mathbf{a}$ - see Worksheet 04 for details of calculation.							
Term 1	0.1703990131						
Term 2	-0.0867316106						
Term 3	0.0269227788						
$\mathbf{a'} \cdot (\mathbf{X'X})^{-1} \cdot \mathbf{a}$	0.1106	calculation verified in Mathematica 3.0.1.1					
$\log_{10} k_a = 0.233255 \log_{10}(k_{o/w}) - 0.005657\ MW - 1.49615$					see Worksheet 04		
$\log_{10}$ of first order absorption rate (k_a)							
Central estimate	-1.84180796375	±	$t_{0.025}$	×	s	×	$(\mathbf{a'} \cdot (\mathbf{X'X})^{-1} \cdot \mathbf{a})^{0.5}$
Lower limit	-2.38007240397	-	2.0560	×	0.787218	×	0.33256578297
Upper limit	-1.30354352353	+	2.0560	×	0.787218	×	0.33256578297
First order absorption rates (antilog or 10^x of above values)							
Central estimate	0.0143943493	$hours^{-1}$					
Lower limit	0.004167999	$hours^{-1}$					
Upper limit	0.0497114552	$hours^{-1}$					

Worksheet 09: Calculation of dermal permeability rate (K_p) in cm/hour for Verbenone.							
Parameters	Value	Units			Reference		
Molecular weight	150.21	g/mole					
$K_{o/w}$ at pH 7	144.9	unitless					
$\log_{10} K_{o/w}$	2.16						
Column vector ***a*** for calculating confidence intervals (see Worksheet 05 for definitions.)							
a_1	1						
a_2	150.21						
a_3	2.16						
Calculation of $\mathbf{a}' \cdot (\mathbf{X}'\mathbf{X})^{-1} \cdot \mathbf{a}$ - see Worksheet 05 for details of calculation.							
Term 1	0.0186064495						
Term 2	-0.007874127						
Term 3	0.004993986						
$\mathbf{a}' \cdot (\mathbf{X}'\mathbf{X})^{-1} \cdot \mathbf{a}$	0.0157	calculation verified in Mathematica 3.0.1.1					
$\log_{10}$ of First order absorption rate							
Central estimate	-2.12258705754	±	$t_{0.025}$	×	s	×	$\mathbf{a}' \cdot (\mathbf{X}'\mathbf{X})^{-1} \cdot \mathbf{a}^{0.5}$
Lower limit	-2.30116070256	-	1.9600	×	0.727129	×	0.12529964086
Upper limit	-1.94401341253	+	1.9600	×	0.727129	×	0.12529964086
First order absorption rates							
Central estimate	0.0075407	cm/hour					
Lower limit	0.0049985	cm/hour					
Upper limit	0.0113759	cm/hour					

Worksheet 10: Summary of chemical specific dermal absorption values used for Verbenone dermal absorption.				
Description	Code	Value	Units	Reference/Source
Zero-order absorption (K_p)				
Central estimate	KpC	0.0075	cm/hour	Worksheet 09, values rounded to two significant figures
Lower limit	KpL	0.005	cm/hour	
Upper limit	KpU	0.011	cm/hour	
First-order absorption rates (k_a)				
Central estimate	AbsC	0.014	$hour^{-1}$	Worksheet 08, values rounded to two significant figures
Lower limit	AbsL	0.0042	$hour^{-1}$	
Upper limit	AbsU	0.05	$hour^{-1}$	

Worksheet 11: Workers: Dermal Exposure Assessments Using Zero-Order Absorption. See section 3.2.2.2 for verbal description.			
Parameter	Value	Units	Source
Density of Verbenone	970	mg/cm^2	Table 2-1
Body weight (*W*)	70	kg	Worksheet 02.BW
Surface Area of hands (*S*)	840	cm^2	Worksheet 02.Hands
Dermal permeability (K_p, cm/hour) [see Worksheet 09]			
Typical	0.0075	cm/hour	Worksheet 09.KpC
Lower	0.005	cm/hour	Worksheet 09.KpL
Upper	0.011	cm/hour	Worksheet 09.KpU

Note that 1 mL is equal to 1 cm^3 and thus mg/mL = mg/cm^3.

Equation (U.S. EPA 1992)

$$K_p \cdot C \cdot Time(hr) \cdot S \cdot \div W = Dose(mg/kg)$$

where:

C = concentration in mg/cm^3 or mg/mL.
S = Surface area of skin in cm^2
W = Body weight in kg.

Wearing Contaminated Gloves for One-Minute

Typical Value: Use typical concentration and central estimate of K_p.
0.0075 cm/hr × 970 mg/cm^3 × 1/60 hr × 840 cm^2 ÷ 70 kg = 1.5e+00 mg/kg [WZHT1M]

Lower Estimate: Use lower limit of K_p.
0.005 cm/hr × 970 mg/cm^3 × 1/60 hr × 840 cm^2 ÷ 70 kg = 9.7e-01 mg/kg [WZHL1M]

Upper Estimate: Use upper range of estimated concentration and upper limit of K_p.
0.011 cm/hr × 970 mg/cm^3 × 1/60 hr × 840 cm^2 ÷ 70 kg = 2.1e+00 mg/kg [WZHU1M]

Wearing Contaminated Gloves for One-Hour

Typical Value: Use typical concentration and central estimate of K_p.
0.0075 cm/hr × 970 mg/cm^3 × 1 hr × 840 cm^2 ÷ 70 kg = 8.7e+01 mg/kg [WZHT1H]

Lower Estimate: Use lower range of estimated concentration and lower limit of K_p.
0.005 cm/hr × 970 mg/cm^3 × 1 hr × 840 cm^2 ÷ 70 kg = 5.8e+01 mg/kg [WZHL1H]

Upper Estimate: Use upper range of estimated concentration and upper limit of K_p.
0.011 cm/hr × 970 mg/cm^3 × 1 hr × 840 cm^2 ÷ 70 kg = 1.3e+02 mg/kg [WZHU1H]

NOTE: If the maximum amount of verbenone in a single pouch were absorbed, the maximum dose would be 63.7 mg/kg. This value is used for the characterization of risk. See Section 3.2.2.2 for a more detailed discussion of the calculations.