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Imidacloprid - Human Health and Ecological Risk Assessment – Final Report

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
a.e.	acid equivalents
AEL	adverse-effect level
a.i.	active ingredient
ALS	acetolactate synthase
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
bw	body weight
CBI	confidential business information
CI	confidence interval
cm	centimeter
CNS	central nervous system
DAA	days after application
DAT	days after treatment
dbh	diameter at breast height
d.f.	degrees of freedom
EC_x	concentration causing X% inhibition of a process
EC_{25}	concentration causing 25% inhibition of a process
EC_{50}	concentration causing 50% inhibition of a process
ExToxNet	Extension Toxicology Network
F	female
FH	Forest Health
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FQPA	Food Quality Protection Act
g	gram
ha	hectare
HQ	hazard quotient
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
k_a	absorption coefficient
k_e	elimination coefficient
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
$K_{o/w}$	octanol-water partition coefficient
K_p	skin permeability coefficient
L	liter
lb	pound
LC_{50}	lethal concentration, 50% kill
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level
m	meter
M	male

mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
mM	millimole
MOS	margin of safety
MRID	Master Record Identification Number
MSDS	material safety data sheet
MW	molecular weight
NCI	National Cancer Institute
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOS	not otherwise specified
NRC	National Research Council
NTP	National Toxicology Program
OC	organic carbon
OM	organic matter
OPP	Office of Pesticide Programs
OPPTS	Office of Pesticide Planning and Toxic Substances
OSHA	Occupational Safety and Health Administration
ppm	parts per million
RBC	red blood cells
RED	re-registration eligibility decision
RfD	reference dose
SERA	Syracuse Environmental Research Associates
t.g.i.a.	technical grade active ingredient
UF	uncertainty factor
U.S.	United States
USDA	U.S. Department of Agriculture
U.S. EPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WHO	World Health Organization
µ	micron
‣	greater than
≥	greater than or equal to
<	less than
≤	less than or equal to
=	equal to
≃	approximately equal to
~	approximately

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

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

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

CONVERSION OF SCIENTIFIC NOTATION

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

EXECUTIVE SUMMARY

OVERVIEW

Imidacloprid is a neurotoxin that is selectively toxic to insects relative to vertebrates and most non-insect invertebrates. This insecticide is used in Forest Service programs to control the hemlock woolly adelgid. The dominant factor in this risk assessment involves the different methods that may be used in applying imidacloprid: tree injection, soil injection, and broadcast applications.

The most common methods in forestry applications are tree injection and soil injections. For soil injection, plausible exposures are below a level of concern by a factor of at least 14 for workers and factors of 30 million to 10 billion for members of the general public. Explicit risk characterizations for tree injection are not made. Tree injection is a very selective application method and levels of exposure for workers and members of the general public are likely to be lower (and probably much lower) than those associated with soil injection. Similarly, no substantial adverse effects for these application methods are anticipated in the ecological risk assessment. In any effective adelgid control program, a plausible adverse effect would be to beneficial insects that prey on adelgids or other similar pest insects. In such cases, effects on these beneficial insects might occur. Field studies have demonstrated adverse effects on some beneficial insects but these effects appear to be transient. The soil injection of imidacloprid is also a relatively specific application method and exposures to most nontarget species will be far below a level of concern with the exception of soil dwelling organisms such as earthworms, soil arthropods, and soil microorganisms. Again, any effects on these species will likely be transient.

More standard methods of pesticide application such as broadcast foliar or broadcast ground applications are less likely to be used in Forest Service programs but these methods are considered in this risk assessment because they may be considered by groups working in cooperation with the Forest Service. In broadcast applications, some plausible exposure scenarios are slightly above the level concern. For workers, the upper range of exposures during the normal broadcast application of either granular or liquid formulations lead to hazard quotients of 1.1. For members of the general public, the highest hazard quotient for non-accidental exposures is 1.5 and this hazard quotient is associated with the upper bound of plausible exposures for the longer-term consumption of contaminated vegetation. The extent to which members of the general public might actually consume vegetation contaminated with imidacloprid is unclear.

Broadcast applications of granular or liquid formulations will result in much greater exposures to a variety of nontarget species. The broadcast application of liquid formulations leads to acute hazard quotients that exceed a level of concern for a large mammal consuming vegetation, a small mammal consuming insects, and large birds consuming grass. For sensitive bird species, the broadcast application of liquid formulations of imidacloprid could be associated with signs of frank toxicity and possibly with substantial mortality after acute exposures. The longer-term consumption of contaminated vegetation by a large bird also exceeds the level of concern. The effects associated with longer-term exposures are regarded as undesirable but the effects, such as weight loss, are not likely to be severe. Imidacloprid is not very toxic to fish, amphibians, and even some aquatic invertebrates. In broadcast applications, however, adverse effects could be seen in some sensitive aquatic invertebrates.

PROGRAM DESCRIPTION

Forest Service uses imidacloprid in the control of the hemlock woolly adelgid (*Adelges tsugae*), a pest of hemlocks (*Tsuga spp.*). The formulations labeled for the control of adelgid species include granules, wettable powders, water soluble pouches, liquids, and capsules. Many different application methods are available for imidacloprid, depending on the nature of the formulations. Tree injections involve the use of specialized application devices to insert imidacloprid (either capsule or liquid formulations) directly into the tree. Similarly, soil injections involve other specialized application devices that insert metered amounts of imidacloprid into the soil, below the soil surface. More standard methods of pesticide application such as broadcast foliar or broadcast ground applications are less likely to be used in Forest Service programs but these methods are considered in the current risk assessment because these application methods might be considered by groups working in cooperation with the Forest Service (other local, state, or federal governmental organizations).

The maximum annual application rate for imidacloprid is 0.5 lb/acre but the maximum rate for a single application is 0.4 lb/acre. Because applications of imidacloprid are very labor intensive, the Forest Service will not apply any imidacloprid formulation more than once per year. Thus, the maximum single application rate considered in this risk assessment is 0.4 lb/acre. Imidacloprid has not been used extensively in past Forest Service programs. Currently, the best estimate is that the Forest Service might use up to 2000 lbs of imidacloprid per year. This use is inconsequential compared to the total agricultural use in the United States (over 60,000 lbs/year). In the southeast region of the United States, however, the use of imidacloprid in forestry applications could be a substantial relative to agricultural use.

HUMAN HEALTH RISK ASSESSMENT

Hazard Identification – Imidacloprid is a neonicotinoid insecticide which produces neurotoxicity through binding or partial binding to specific areas of the nicotinic acetylcholine receptor. Acetylcholine is an important neurotransmitter in both insects and mammals; it is released at the nerve synapse in response to a membrane depolarization which is the hallmark of nerve transmission. There are different types of acetylcholine receptors. One type of receptor is called the nicotinic acetylcholine receptor (nAChR), which is activated by nicotine. Nicotine binds at or near the location where acetylcholine binds, causing the cascade of events leading to nerve transmission. Although imidacloprid activates nAChR, it is important to note that it does so in a manner fundamentally different from nicotine. This is important because, unlike nicotine, imidacloprid is more toxic to insects than to mammals.

Imidacloprid and its nitrosoimine metabolite (WAK 3839) have been well studied in rats, mice and dogs. In mammals, the primary effects following acute high-dose oral exposure to imidacloprid are mortality, transient cholinergic effects (dizziness, apathy, locomotor effects, labored breathing) and transient growth retardation. Exposure to high doses may be associated with degenerative changes in the testes, thymus, bone marrow and pancreas. Cardiovascular and hematological effects have also been observed at higher doses. The primary effects of longer term, lower-dose exposure to imidacloprid are on the liver, thyroid, and body weight (reduction). Low- to mid-dose oral exposures have been associated with reproductive toxicity, developmental retardation and neurobehavioral deficits in rats and rabbits. Imidacloprid is neither carcinogenic in laboratory animals nor mutagenic in standard laboratory assays.

The nitrosoimine metabolite (WAK3839), which is an impurity of technical-grade imidacloprid, does not appear to be produced *in vivo* except after long-term high-dose exposure. The nitrosoimine metabolite is not mutagenic, and is of equivalent or lower toxicity than that of imidacloprid on the basis of acute and subchronic toxicity.

Exposure Assessment – The exposure assessments for this risk assessment are detailed in four sets of worksheets that accompany this risk assessment:

broadcast applications of liquid formulations on clay or loam soils;
broadcast applications of granular formulations on clay or loam soils;
soil injections in clay or loam soils;
applications (any method) to predominantly sand soils.

No quantitative exposure assessments are given for tree injection of imidacloprid; this application method is extremely specific to the targeted species (adelgids) and the plant to be protected (hemlocks). There is no apparent basis for asserting that human exposures due to tree injection are likely to be substantial, and there are no methods and no information sufficient to quantify the exposures except to suggest that the exposures will be less than those associated with other application methods. A similar problem exists for workers applying imidacloprid by soil injection. While it seems plausible that soil injection applications will lead to exposures that are less than those associated with more standard broadcast applications, very little information is available to substantiate this supposition. Thus, for workers involved in soil injection application, the exposure assessment is based on exposure rates associated with backpack applications. These will almost certainly overestimate worker exposures during soil injection and these overestimates may be extreme.

For both workers and the general public, exposure assessments are presented for both aerial and ground broadcast applications. These applications are included at the request of the Forest Service in response to comments from cooperators who may wish to consider these application methods. In Forest Service programs, however, only tree injection and soil injection applications are anticipated.

Central estimates of exposure for workers are approximately 0.005 mg/kg/day for aerial and backpack workers and about 0.009 mg/kg/day for broadcast ground spray workers. Upper ranges of exposures are approximately 0.06 mg/kg/day for backpack and aerial workers and about 0.03 mg/kg/day for broadcast ground spray workers. All of the accidental exposure scenarios for workers involve dermal exposures and these accidental exposures lead to estimates of dose that are comparable to or substantially below the general exposure estimates for workers.

For the general public, the range for acute exposures is about 0.00000001 mg/kg bw to about 0.3 mg/kg bw. For soil injection applications, all non-accidental exposures are extremely low. For all application methods, the upper range of exposure is associated with scenarios involving the accidental spill of imidacloprid into a relatively small body of water.

For chronic (long-term) exposures, the modeled exposures are much lower than for acute (short-term) exposures. The highest chronic exposure is about 0.09 mg/kg/day and is associated with the consumption of contaminated broadleaf vegetation after broadcast applications of liquid

formulations. For soil injection, a method that may be used in Forest Service programs, the highest chronic exposure is 0.000001 mg/kg/day and is associated with the consumption of contaminated water after application to sandy soil. However, the Forest Service does not anticipate applying imidacloprid to predominantly sandy soils and the corresponding exposures associated with clay or loam soils are negligible.

Dose-Response Assessment – Following standard practices for Forest Service risk assessments, reference values (RfDs) available from the U.S. EPA are adopted. U.S. EPA has derived a chronic RfD for imidacloprid of 0.057 mg/kg/day. This chronic RfD is well-documented and is used directly for all longer term exposures to imidacloprid. This value is based on a NOAEL of 5.7 mg/kg/day in rats and an uncertainty factor of 100 – two factors of 10 for interspecies and intraspecies variability. U.S. EPA has derived an acute RfD for imidacloprid of 0.14 mg/kg/day. This value is based on a LOAEL of 42 mg/kg in rats and an uncertainty factor of 300 - a factor of three for extrapolating NOAEL from LOAEL, and two factors of 10 for interspecies and intraspecies variability.

Risk Characterization – The risk characterization for potential human health effects is influenced by the application method. For soil injection and tree injection (i.e., the application methods that are likely to be used by the Forest Service), the risk characterizations for workers and members of the general public are reasonably unequivocal. None of the acute or longer term hazard quotients exceed 1, the level of concern. For members of the general public, the hazard quotients are below the level of concern by factors of 30 million to 10 billion. Workers are likely to be subject to higher levels of exposure. Nonetheless, the highest hazard quotient for workers involved in soil injection is below the level of concern by a factor of about 14. Explicit risk characterizations for tree injection are not made. This is a very selective application method and levels of exposure for workers and members of the general public are likely to be lower (and probably much lower) than those associated with soil injection.

Although the Forest Service does not anticipate using broadcast applications of imidacloprid, these application methods are considered in this risk assessment because other organizations working in cooperation with the Forest Service may consider using broadcast applications of either granular or liquid formulations. In broadcast applications, some exposure scenarios result in modest excursions about the level concern. For workers, the upper range of exposures during the normal broadcast application of either granular or liquid formulations lead to hazard quotients of 1.1. For members of the general public, the highest hazard quotient for non-accidental exposures is 1.5 and this hazard quotient is associated with the upper bound of plausible exposures for the longer-term consumption of contaminated vegetation. Whether members of the general public might actually consume vegetation contaminated with imidacloprid is unclear. Broadcast applications of imidacloprid will not be applied intentionally to crops or other types of vegetation that humans might consume. The intent of broadcast applications will be to apply the imidacloprid to the target vegetation – i.e., hemlocks. Human consumption of contaminated vegetation would be unintentional and probably incidental.

Hazard quotients for accidental exposures associated with spills into a small body of water result in hazard quotients with upper bounds that range from 1.1 (adult male consuming fish) to 15 (a child consuming 1 liter of contaminated water). The amounts spilled are set at the amounts required to treat from one acre (0.4 lbs) to100 acres (40 lbs). These assumptions are completely

arbitrary and may be unrealistic. Given the relatively small areas that the Forest Service treats with imidacloprid, it seems highly unlikely that the amount required to treat 100 acres would be assembled in one container or vehicle and would then be spilled into a small pond. This exposure scenario is intended simply to illustrate the different consequences of spilling different amounts of imidacloprid. Any reasonable assessment of risk would need to be based on site-specific information of an actual spill.

ECOLOGICAL RISK ASSESSMENT

Hazard Identification – The toxicity of imidacloprid has been well-studied in mammals, birds, terrestrial invertebrates and aquatic organisms, and the mechanism of action is fairly well known. In all species, the toxicity of imidacloprid metabolites is equivalent to or less than that of the parent compound. The nitrosoimine metabolite, a contaminant of imidacloprid preparations (as much as 30%) and a product of imidacloprid metabolism, is of low toxicity to mammals. The predominant metabolites associated with toxicity in insects are olefinic-, dihydroxy- and hydroxy-imidacloprid.

In mammals, the primary toxic effects of imidacloprid are on body weight and the thyroid. In birds, imidacloprid causes neurotoxicity and adverse effects on hatchling growth, and there is evidence that birds learn to avoid imidacloprid-treated seed. Birds appear to be more sensitive to imidacloprid than mammals.

The body of literature on the effects of imidacloprid on insects is large and diverse. There is a general pattern of toxicity following imidacloprid exposure, involving an immediate onset of neurotoxicity, followed by a delayed mortality, usually 4 hours to several days after exposure. Evidence suggests that unchanged imidacloprid may be responsible for the initial neurotoxicity, while the olefinic, hydroxy- and dihydroxy- metabolites which appear at approximately 4 hours post-exposure may be responsible for mortality.

The effects of imidacloprid on beneficial predatory arthropods appear to depend upon the species, and the conditions and rate of application. The parasitic hymenopterans appear to be most sensitive, while ants are most tolerant. In honey bees, imidacloprid at very low doses has been shown to cause mortality and adverse effects on laboratory-conditioned behavioral responses associated with feeding. However, adverse impacts of imidacloprid on foraging and colony vitality under field conditions have yet to be demonstrated. In fact, key studies suggest that imidacloprid may not induce the same learned avoidance behavior in honey bees that have been demonstrated in birds.

Fish, amphibians and aquatic algae are less sensitive to imidacloprid than certain aquatic invertebrates in terms of survival and growth. Among aquatic invertebrates, arthropods such as chironomid and mysid species are extremely sensitive to imidacloprid exposure, with observed adverse effects on survival, growth and reproductive success.

Exposure Assessment – As in the human health risk assessment and for the same reasons, the quantitative exposure assessments are detailed in four EXCEL workbooks by application method and soil type:

broadcast applications of liquid formulations on clay or loam soils;

broadcast applications of granular formulations on clay or loam soils;
soil injections in clay or loam soils;
applications (any method) to predominantly sand soils.

While this approach is more complicated than that taken in most Forest Service risk assessments, it is necessary because exposures vary substantially with the different application methods for imidacloprid. For tree injection, no quantitative exposures are presented. For the same rationale articulated in the human health risk assessment, there is no basis for asserting that substantial exposures to most terrestrial organisms are plausible from tree injection. A major exception, of course, is the target species (adelgids) and other insects that might feed on treated trees. Additional and perhaps significant exposures are likely to some beneficial insects that prey on adelgids and other insect pests of hemlocks. Potential risks to these species are characterized using the available field or field simulation studies.

For soil injection applications as well as broadcast applications, exposures to soil organisms are likely. Exposures to other terrestrial animals from soil injection will primarily involve contaminated water. These exposures are summarized in the workbooks for applications to loam or clay soils and applications to predominantly sandy soils. The estimated concentrations of imidacloprid in surface water are similar for sandy soils after applications by broadcast or soil injection.

While the Forest Service does not anticipate using broadcast applications of liquid or granular formulations, these application methods are covered in the current risk assessment. For broadcast applications, terrestrial animals might be exposed to any applied pesticide from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation. As with the human health exposure assessment, two sets of exposure scenarios are provided in two separate EXCEL workbooks, one for liquid formulations and the other for granular applications. These exposure assessments are generally similar, but some of the computational details vary because of differences between granular and liquid formulations. In addition, there is a substantial difference in residue rates on contaminated vegetation, with much higher residues expected after foliar application of liquid formulations compared to those expected after soil application of granular formulations. For aquatic species, the concentrations in water are identical to those used in assessing exposures to both terrestrial wildlife and humans.

Dose-Response Assessment – The available toxicity data on nontarget species support separate dose-response assessments in six classes of organisms: terrestrial mammals, birds, non-target terrestrial invertebrates, fish, aquatic invertebrates, and aquatic algae. Different units of exposure are used for different groups of organisms depending on how exposures are likely to occur and how the available toxicity data are expressed.

On the basis of both acute and chronic toxicity, the order of sensitivity to imidacloprid among terrestrial organisms is honey bees (most sensitive), followed by birds, and then mammals (least sensitive). The acute and chronic NOAEL values are: 0.013 mg/kg and 0.010 mg/kg/day for honey bees; 3 mg/kg and 0.3 mg/kg/day for birds; and 5.7 mg/kg/day and 0.14 mg/kg for mammals.

Due to the number of studies in the open literature which attempt to assess the potential effects of imidacloprid on beneficial predatory arthropods other than honey bees, there are values for beneficial predators, which are presented in terms of application rate. These values are used to qualify and refine conclusions based on the the standard bioassay studies using honey bees.

Both acute and chronic toxicity values for aquatic species indicate a large difference between fish and certain sensitive aquatic invertebrates. For fish, the acute NOAEC values are 25 mg/L and 50 mg/L for sensitive and tolerant species, respectively. For invertebrates, the corresponding acute NOAEC values are 0.00035 mg/L and 145 mg/L. For fish, a chronic NOAEC of 9.8 mg/L is available from a chronic life-stage study. Chronic NOAEC values of 0.000163 mg/L and 1.8 mg/L are used for sensitive and tolerant aquatic invertebrates, respectively. Toxicity values of 6.69 mg/L (sensitive) and 119 mg/L (tolerant) are used for aquatic algae. Because of the short life-cycle of individual algal cells, the relatively short-term bioassays in algae (i.e., 96 to 120 hours) are applied to both acute and longer-term concentrations for the characterization of risk.

On the basis of acute toxicity, amphibians are less sensitive than mammals, fish, and sensitive aquatic invertebrates. Acute NOEC values of 30 mg/L and 101.2 mg/L are used in this assessment for sensitive and tolerant amphibian species, respectively. For longer-term exposures, NOEC values of 17.5 mg/L and 88 mg/L are used for sensitive and tolerant species, respectively.

The risks associated with metabolites of imidacloprid are not addressed directly or quantitatively in this assessment. In mammals, fish, and aquatic invertebrates, no metabolite tested was shown to cause toxicity at lower concentrations than the parent imidacloprid compound. In insects the olefin, 5-hydroxy and 4,5-di-hydroxy-metabolites were shown to be active in causing toxicity at or below the concentrations at which imidacloprid causes adverse effects. Although it has been hypothesized that these metabolites might be responsible for the delayed mortality observed in many acute studies with insects following exposure to imidacloprid, it is assumed that any benchmark values protective of the adverse effects of imidacloprid will also be protective of it's metabolites. Therefore, toxicity values for individual imidacloprid residues are not derived in this assessment.

Risk Characterization – As with the human health risk assessment, the risk characterization for imidacloprid is dependent on the application method. The Forest Service will typically restrict applications of imidacloprid to either tree injection or soil injection in clay or loam soils. Neither of these application methods are likely to cause adverse effects in nontarget species. Broadcast applications of imidacloprid may be considered by some groups working in cooperation with the Forest Service. Broadcast applications will result in higher exposures to nontarget species and some adverse effects are plausible.

Tree injection of imidacloprid is highly specific and will not result in substantial exposures to nontarget species. The only plausible exception would be beneficial insects that prey on adelgids or other similar pest insects. In such cases, effects on these beneficial insects might occur. Field studies have demonstrated adverse effects on some beneficial insects but these effects tend to be transient.

Soil injection of imidacloprid is also a relatively specific application method and exposures to most nontarget species will be far below a level of concern. An obvious exception, however, involves soil dwelling organisms such as earthworms, soil arthropods, and soil microorganisms. After soil injection, concentrations of imidacloprid will be in the range of soil concentrations that have been shown to cause sperm deformity in earthworms. In addition, field studies have demonstrated decreases in earthworm populations after applications of imidacloprid comparable to rates used in Forest Service programs. This effect, however, appear to be transient. There is little indication that imidacloprid is likely to cause adverse effects on soil microorganisms. Concentrations of imidacloprid could approach or somewhat exceed those associated with decreases in populations of soil fungi (but not soil bacteria). Again, these effects will be transient and concentrations of imidacloprid in soil will decrease to levels below those that might be associated with effects in fungi.

Broadcast applications of granular or liquid formulations will result in much greater exposures to a wider variety of nontarget species than will the selective applications discussed above. The greatest difference between granular and liquid formulations will involve residues on vegetation and insects. Liquid formulations are likely to result in substantially greater residues than granular formulations. The broadcast application of liquid formulations lead to acute hazard quotients that exceed a level of concern for a large mammal consuming vegetation (HQ=1.4), a small mammal consuming insects (acute HQ=2), and large birds consuming grass (HQ=10). For sensitive bird species, the broadcast application of liquid formulations of imidacloprid could be associated signs of frank toxicity and possibly with substantial mortality after acute exposures.

The longer-term consumption of contaminated vegetation by a large bird also exceeds the level of concern (HQ=1.7). The effects associated with longer-term exposures are regarded as undesirable but the effects, such as weight loss, are not likely to be severe. There is no indication that frank adverse effects such as obvious debilitation or mortality would be observed.

Imidacloprid is not very toxic to fish, amphibians, and even some aquatic invertebrates. No effects on any aquatic species are likely after either tree injection or soil injection applications to predominantly clay or loam soils. In addition, worst-case estimates of peak or longer-term exposures from broadcast applications indicate that adverse effects are not likely to be observed in aquatic vertebrates. Differences between sensitive and tolerant aquatic invertebrate species are substantial, spanning a factor of over 400,000 for acute NOEC values and over 11,000 for longer-term NOEC values. Depending on the application method and soil type, hazard quotients for sensitive aquatic invertebrates could range from about 2 to over 80.

As in the human health risk assessment, the ecological risk assessment uses a scenario for an accidental spill that involves the contamination of a small body of water with 0.4 lb to 40 lbs of imidacloprid. Over this range, the hazard quotients for sensitive aquatic invertebrates are extraordinarily high, ranging from about 500 to over 50,000. While the likelihood and plausibility of such spills may be remote, these hazard quotients clearly suggest that the greatest risk in the event of an accidental spill will be to aquatic invertebrates. As with fish and amphibians, tolerant aquatic invertebrates are not at risk in the event of an extreme spill.

1. INTRODUCTION

The USDA Forest Service uses imidacloprid in the control of the hemlock woolly adelgid (*Adelges tsugae*), an insect pest of hemlocks (*Tsuga spp.*) in the eastern United States (USDA/FS 1994; Webb et al, 2003). This document provides risk assessments for human-health effects and ecological effects to support an assessment of the environmental consequences of this use.

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

Although this is a technical support document and addresses some specialized technical areas, an effort was made to ensure that the document can be understood by individuals who do not have specialized training in the chemical and biological sciences. Certain technical concepts, methods, and terms common to all parts of the risk assessment are described in plain language in a separate document (SERA 2001). Technical terms that are common to this and many other risk assessments conducted for the Forest Service are available on the internet at www.sera-inc.com.

The human health and ecological risk assessments presented in this document are not, and are not intended to be, comprehensive summaries of all of the available information. Much of the published literature on imidacloprid is summarized by WHO (2001) and the U.S. EPA has evaluated the toxicity of imidacloprid under the requirements of the Food Quality Protection Act (FQPA) (U.S. EPA/OPP 1998) and in the development of pesticide tolerances (U.S. EPA/OPP 2003; U.S. EPA/OPP 2005a,b). Other reviews and evaluations of the potential risks associated with the use of imidacloprid have been presented by Cox (2001), Dikshit and coworkers (Dikshit and Lal 2002; Dikshit et al. 2003), Graney and Fischer (1992a,b), Schmuck et al. (2001), Toll and Fischer (1993), and Yen and Wendt (1993). These reviews were consulted in the preparation of this risk assessment and the most relevant studies are summarized in the appendices included with this risk assessment. Nonetheless, the discussions in Section 3 (Human Health Risk Assessment) and Section 4 (Ecological Risk Assessment) focus on those studies that have a direct impact on the risk characterization for imidacloprid.

A complete search of the U.S. EPA FIFRA/CBI files was conducted. These are studies that are required by the U.S. EPA to support the registration of pesticides. These studies are typically conducted either by the company seeking registration of the pesticide or by commercial testing facilities under funding by the company seeking registration of the pesticide. These studies are preferred by the U.S. EPA for pesticide registration because they follow guidelines established by the U.S. EPA (e.g., http://www.epa.gov/OPPTS_Harmonized/). A total of 903 submissions were identified. Of these, 311 studies potentially relevant to this risk assessment were identified. Under the Freedom of Information Act (FOIA), SERA requested and received a total of 213 studies. The difference between the 311 potentially relevant studies and the 213 studies received

through FOIA related to limitations on FOIA requests. Only studies conducted after 1986 and studies relating to toxicity or environmental fate are eligible for release under FOIA. Studies on the identity of impurities, inerts, adjuvants, and manufacturing processes are considered proprietary and are not eligible for release under FOIA. Full text copies of the studies that could be released under FOIA were kindly provided by the U.S. EPA Office of Pesticide Programs. These studies were reviewed, are discussed in Sections 3 and 4 as necessary, and synopses of the most relevant studies are provided in the appendices to this document.

The Forest Service will update this and other similar risk assessments on a periodic basis and the Forest Service welcomes input from the general public on the selection of studies included in the risk assessment. This input is helpful, however, only if recommendations for including additional studies specify why and/or how the new or not previously included information would be likely to alter the conclusions reached in the risk assessments.

Almost no risk estimates presented in this document are given as single numbers. Usually, risk is expressed as a central estimate and a range, which is sometimes very large. Because of the need to encompass many different types of exposure as well as the need to express the uncertainties in the assessment, this risk assessment involves numerous calculations. Most of the calculations are relatively simple, and the very simple calculations are included in the body of the document.

Some of the calculations, however, are cumbersome. For those calculations, EXCEL worksheets are included as attachments to this risk assessment. The worksheets provide the detail for the estimates cited in the body of the document. The worksheets for imidacloprid are contained in EXCEL workbooks that accompany this risk assessment. Documentation for the use of these worksheets is presented in SERA (2005). The worksheets are an integral part of the risk assessment. The worksheets are designed to isolate the large number of calculations from the risk assessment narrative. In general, all calculations of exposure scenarios and quantitative risk characterizations (i.e., hazard quotients) are derived and contained in the worksheets. The rationale for the calculations as well as the interpretation of the hazard quotients are contained in this risk assessment document.

Four workbooks (sets of worksheets) are included with this risk assessment: broadcast applications of liquid formulations on clay or loam soils (Attachment 1), broadcast applications of granular formulations on clay or loam soils (Attachment 2), soil injections in clay or loam soils (Attachment 3), and applications by any method to predominantly sand soils (Attachment 4). The rationale for each of these separate workbooks is discussed in Section 2.3.4 (Relationship of Application Methods to Workbooks) of the program description. Additional details are provided in Section 3.2 (Exposure Assessment for the Human Health Risk Assessment) and Section 4.2 (Exposure Assessment for the Ecological Risk Assessment).

2. PROGRAM DESCRIPTION

2.1. OVERVIEW

The Forest Service uses imidacloprid in the control of the hemlock woolly adelgid (*Adelges tsugae*), a pest of hemlocks (*Tsuga spp.*). The formulations labeled for the control of adelgid species include granules, wettable powders, water soluble pouches, liquids, and capsules. Many different application methods are available for imidacloprid, depending on the nature of the formulations. The most common methods used in forestry applications are tree injection and soil injection. Tree injection involves the use of specialized application devices to insert imidacloprid (either capsule or liquid formulations) directly into the tree. Similarly, soil injection involves other specialized application devices that insert metered amounts of imidacloprid into the soil, below the soil surface. More standard methods of pesticide application such as broadcast foliar or broadcast ground applications are unlikely to be used in Forest Service programs but these methods are considered in the current risk assessment. The maximum annual application rate for imidacloprid is 0.5 lb/acre but the maximum rate for a single application is 0.4 lb/acre. Because applications of imidacloprid are very labor intensive, the Forest Service will not apply any imidacloprid formulation more than once per year. Thus, the maximum application rate considered in this risk assessment is 0.4 lb/acre. Imidacloprid has not been used extensively in past Forest Service programs. Currently, the best estimate is that the Forest Service might use up to 2000 lbs of imidacloprid per year. This use is inconsequential compared to the total agricultural use in the United States (over 60,000 lbs/year). In the southeast region of the United States, however, the use of imidacloprid in forestry applications could be substantial relative to agricultural use.

2.2. CHEMICAL DESCRIPTION AND COMMERCIAL FORMULATIONS

Imidacloprid is a systemic insecticide that is used to control pest insects on vegetation. A very large number of imidacloprid formulations are available (e.g., http://www.cdms.net/manuf/default.asp and www.greenbook.net) for the control of a large number of pest insects – e.g., aphids, Japanese beetles, lacebugs, leaf beetles, leafhoppers, leafminers, white flies etc.

This risk assessment is focused on Forest Service uses in the control of the hemlock woolly adelgid (*Adelges tsugae*). The hemlock woolly adelgid is a pest of hemlocks (*Tsuga spp.*). The insects suck sap from growing hemlock twigs. In severe infestations, the resulting loss of needles and twigs can damage the health of the tree (Webb et al, 2003). While the hemlock woolly adelgid can be found in both the Pacific Northwest and the Eastern United States, damage to hemlocks appears to be most severe in the East (Hoover 2000).

As discussed further in Section 3.1.6 (Effects on the Nervous System) and Section 4.3.2.3 (Terrestrial Invertebrates), imidacloprid is a neurotoxic agent that interferes with a neural pathway that is more important in insects than mammals. Imidacloprid is applied to either soil or foliage and is systematically taken up by the plant. When a sucking insect such as the hemlock woolly adelgid feeds on the plant, it consumes imidacloprid residues from the plant and is killed. When applied to foliage, imidacloprid acts mainly as a contact insecticide (Carlin 2005).

A general description of the chemical and physical properties of imidacloprid is presented in Table 2-1. The commercial formulations of imidacloprid that are labeled for the control of adelgid species or are known to be used in Forest Service programs for the control of the hemlock woolly adelgid are identified in Table 2-2. The patent for imidacloprid will expire in 2005 and it is likely that other formulations, essentially identical to those summarized in Table 2-2 will become available. When some of these newer formulations become available, they could be used in Forest Service programs (Onken 2005).

The formulations labeled for the control of adelgid species include granules (Marathon 1%, Merit 2.5 G), wettable powders (Marathon WP, Merit 75 WP), water soluble pouches (Merit 75 WSP), liquids (Marathon II, Marathon F, Pointer), and capsules (Imicide, IMA-jet). As discussed further in Section 2.3, these formulations are applied using a variety of different methods depending on the formulation and application site.

The identity of all inerts for each formulation has been disclosed to the U.S. EPA as part of the registration process (Arborsystems 1995; Bayer Environmental 2004; Davis 1995, 2002; Fontaine 1992a to g; Fontaine 1994a,b,c; Fontaine 1996; Fontaine 1997a,b; Fontaine 1999; Lewis And Harrison 2004; Mitchell 2001; Mitchell 2004a,b; Talbott 1991a to i). As indicated in Section 1, these studies are considered proprietary, are not eligible for release under FOIA, and have not been reviewed as part of the current risk assessment. Nonetheless, as summarized in Table 2-3, some information is available to the public on the inerts contained in the formations of imidacloprid that are covered in this risk assessment. This information comes primarily from the Material Safety Data Sheets for the formulations. In addition, the Northwest Coalition for Alternatives to Pesticides (NCAP) has obtained information on the identity of other inerts from U.S. EPA under the Freedom of Information Act and has listed this information on the NCAP web site (http://www.pesticide.org/FOIA/). The potential contribution of the inerts to the toxicity of formulations is discussed further in Section 3.1.14 (Inerts and Adjuvants).

2.3. APPLICATION METHODS

2.3.1. Soil Applications

As summarized in Table 2-2, imidacloprid may be applied to soil by broadcast application, mechanical incorporation, soil drench, or soil injection. All of these application methods involve an attempt to achieve a concentration of imidacloprid in the soil. As noted above, the imidacloprid will then be transported from the roots to the twigs where the target insects will feed.

Soil broadcast applications involve spreading the formulation under the plants to be protected. Either rainfall or direct irrigation may be used to “activate” the imidacloprid – i.e., to transport the imidacloprid from the surface of the soil into the root zone of the plant. Soil broadcast applications may be made with granular formulations (Marathon 1% G; Merit 2.5 G), wettable powders (Marathon WP), or liquid formulations (Marathon II). This type of application typically involves a standard expression of application rate – i.e., lb/acre – as discussed further in Section 2.4.

Soil drench involves a process similar to that of soil broadcast applications. The formulation is applied to the soil (either as a granular or liquid) and then watered in. This application method is recommended for Marathon WP, Merit 2F, Marathon II, Merit 75 WP, and Merit 75 WPS. The product labels for some formulations suggest that soil drench will be used primarily in nursery environments rather than general forestry. For example, soil drench is recommended for Marathon WP in adelgid control for containerized plants. Other formulations – e.g., Merit 2F – recommend soil drench for trees. All of the soil drench applications require a prescribed amount of water, typically on the order of 10 gallons per 1000 square feet. This corresponds to an irrigation of about 0.041cm of water [10 gal/ (31.62 ft x 31.62 ft) = 37.85 L/963.93cm x 963.93cm = 37,850 cm^3 / 929,161 cm^2 = 0.040736 cm]. The requirement for irrigation limits the use of this application method to areas where water is readily available. Typically, the application rate for soil drench is expressed in units of amount of formulation per unit of trunk diameter or shrub height. Thus, estimates of standard application rates in units of pounds per acre are uncertain.

Although the Forest Service did not use soil drench applications in the 2005 suppression program, soil drench of imidacloprid could be used in treating isolated high-value hemlocks located on developed areas. Since this method would only be used to treat a very small subset of isolated trees within a given acre, it is impossible to determine application rates in lbs a.i. per acre (Carlin 2005).

The product labels for some formulations – i.e., Marathon 1%, Marathon 60 WP, – indicate that the product may be applied by mechanical soil incorporation. In other words, some mechanical means is used to physically mix the formulated product with the soil. This application method is employed in nursery environments and has no substantial field use. For this application method, the application rate is typically expressed as a target concentration in soil – e.g., pounds of formulation per unit of soil volume.

All of the Merit formulations listed in Table 2-2 as well as Marathon 60 WP and Marathon II are labeled for soil injection. This type of application involves using a solution or suspension of the formulation and placing the liquid in an injection pump designed to insert or inject a metered volume of the liquid into the soil, typically to a depth of about two to six inches. Soil injections may be made using a circle system, basal stem injection, or a combination of these patterns. The number of injections that are made and the volume of material that is injected into the soil varies with the size of tree. For example, Marathon 60 WP is injected at a rate of 20 grams of formulation per 8 to 16 inches of cumulative tree diameter. Because soil injection does not require the use of artificial irrigation, this method may be used in forestry (as opposed to nursery) applications and is one of the most important application methods for Forest Service programs.

2.3.2. Foliar Broadcast Applications

Provado 1.6 is the only formulation of imidacloprid that is labeled for aerial applications in the control of adelgids. Aerial applications are made under meteorological conditions that minimize the potential for spray drift. The product label for Provado 1.6 specifies that aerial applications should be made only when the wind speed is greater than 3 mph and less than 15 mph. While no droplet size specification is given in the product label, the label does specifically note that small droplets (i.e., <150 to 200 microns) will favor drift. In practice, the Forest Service considers droplets less than 100 microns to be "small" in terms of favoring drift. Since hemlock typically occurs in small patches and in riparian areas, aerial applications of imidacloprid are likely to very limited and small in size (Onken 2005). Thus, while aerial applications are covered in the current risk assessment in the event that the Forest Service may need to consider this option, aerial applications are not likely to be used in Forest Service programs.

Provado 1.6 may also be applied in broadcast or directed foliar applications using ground equipment. Ground foliar broadcast applications involve spray equipment mounted on tractors or trucks and airblast sprayers may be used to apply imidacloprid to the tree canopy. As with aerial applications, a ground broadcast application is considered in the current risk assessment but it is not likely that Provado 1.6 would be used in foliar ground broadcast applications in Forest Service programs (Carlin 2005).

In typical Forest Service risk assessment for herbicides, the assumption is made that about 8 acres will be ground-mechanically treated in a 45-minute period (approximately 11 acres/hour) with approximately 200 gallons of the pesticide mixture (270 gallons/hour) and that some special truck mounted spray systems may be used to treat up to 12 acres in a 35-minute period with approximately 300 gallons of herbicide mixture (about 21 acres/hour and 510 gallons/hour) (USDA 1989; pp. 2-9 to 2-10). These large scale broadcast applications, however, are not applicable to imidacloprid. For calculating worker exposures, the maximum area that would be treated in a single day is taken as 200 acres (Onken 2005).

2.3.3. Tree Injection

Two formulations of imidacloprid, Imicide and IMA-jet, may be used in Forest Service programs only in tree injections. Imicide is a capsule formulation – i.e., the liquid insecticide is contained within a capsule. Holes with a diameter of about 11/64 inch are drilled into the tree at a slight downward angle to a depth of about 3/8 to ½ inch. The holes are drilled about 6 to 8 inches above the ground. The number of holes per tree depends on the tree diameter. The capsule is inserted into these holes and into the conductive xylem tissue of the tree and is then ruptured. The liquid insecticide is then rapidly absorbed into the tree and translocated to the branches and needles. IMA-jet is injected into tree roots or into trunk tissue immediately above the trunk flare. The Arboplug is a self-sealing cylindrical container that can be injected directly into tree tissue (http://arborjet.com/products/arborplug.htm). The Arborplug is set into 5/8" deep holes drilled into the sapwood. The infusion process is initiated by piercing an internal septum in the Arborplug. For both formulations, the number of injections and volume of formulation are dependent on the size of the tree. A third injection formulation, Pointer, is available but will not be used in Forest Service programs (Onken 2005).

2.3.4. Relationship of Application Methods to Workbooks

This risk assessment considers a larger number of application methods than are typical in most Forest Service risk assessments (Section 2.3.4). This complicates the exposure assessments and requires a more elaborate set of worksheets than are typically included with Forest Service risk assessments. As noted in the introduction (Section 1), this risk assessment is accompanied by four EXCEL workbooks:

broadcast applications of liquid formulations on clay or loam soils (Attachment 1),
broadcast applications of granular formulations on clay or loam soils (Attachment 2),
soil injections in clay or loam soils (Attachment 3),
applications (any method) to predominantly sand soils (Attachment 4).

Note that no worksheets are included for tree injection. Although tree injection is likely to be a common method used in forestry applications, tree injection is a very targeted application method. Consequently, most of the exposure scenarios used in Forest Service risk assessments do not apply to tree injection applications (e.g., direct spray of animals or vegetation). For other scenarios that may apply (e.g., worker exposure, exposure to nontarget insects), the available data are not adequate to support quantitative exposure assessments. Consequently, the risks associated with tree injection applications are discussed qualitatively in the risk characterizations for the human health (Section 3.4) and ecological effects (Section 4.4).

Of the remaining application methods, soil injection in predominantly clay or loam soils is the application method that is most likely to be used in Forest Service programs (Attachment 3). As with tree injections, many of the standard exposure scenarios used in Forest Service risk assessments, such as those associated with spray and drift, are not applicable to this application method. Nonetheless, estimates are made of some worker exposures as well as exposures associated with contaminated surface water. As detailed in Section 3.2.3.4, contamination of surface water from soil injection in predominantly clay or loam soils is likely to be negligible except in cases of accidental spills, the plausibility of which may be remote.

Two workbooks are included for broadcast applications to predominantly clay or loam soils: one for liquid formulations (Attachment 1) and the other for granular formulations (Attachment 2). Both workbooks consist of a standard set of exposure scenarios that are used in most Forest Service risk assessments and include both ground and aerial broadcast. The Forest Service, however, does not intend to use any form of broadcast application of liquid formulations (i.e., foliar spray) for adelgid control. As detailed in this risk assessment, foliar spray has a relatively high potential for contamination of surface water. In addition, foliar spray in not effective for adelgid control (Cowles 2005). The information on aerial applications is included in the workbooks solely to illustrate the consequences of using aerial application methods, which might be considered in other programs for other pest species. Thus, aerial applications are discussed briefly in the risk characterization sections of this document but are not used directly to assess the consequences of using imidacloprid for adelgid control in the current risk assessment. Soil applications of granular or liquid imidacloprid formulations may play a role in some Forest Service programs or programs conducted by Forest Service cooperators (i.e., groups working in

coordination with the Forest Service on adelgid control programs) (Carlin 2005). Thus, these applications are considered in the risk characterization for the current risk assessment.

All of the above workbooks are limited to applications of imidacloprid to soils that consist predominantly of clay or loam. The Forest Service does not anticipate applications of imidacloprid to predominantly sandy soils (Mistretta 2005). A fourth workbook, however, is provided to illustrated the potential consequences of applying imidacloprid to predominantly sandy soils (Attachment 4). With the exception of tree injection, which has a very low potential for water contamination, applications of imidacloprid to sandy soils lead to very similar estimates of potential concentrations of imidacloprid in ambient water regardless of the specific application method – i.e., soil injection or broadcast applications of granular or liquid formulations (Section 3.2.3.4). The soil type, however, has no significant impact on estimates of worker exposure or exposures associated with contaminated vegetation. Thus, the workbook on applications to sandy soils is a subset of worksheets limited to estimated concentrations in surface water. Other potentially relevant exposures (e.g., worker exposures or exposures associated with contaminated vegetation) will depend on the type of formulation (liquid or granular) and these exposures are included in the workbooks for the application of liquid and granular formulations to clay or loam soils.

Lastly, it should be noted that some of the data available on imidacloprid do not lend themselves to standard numeric expressions of risk. For example, the available data on some nontarget insects are not readily expressed as standard hazard quotients – i.e., ratios of some estimate of exposure to a toxicity value. Such data are discussed qualitatively in the appropriate sections of the risk characterization but are not included in the worksheets.

2.4. MIXING AND APPLICATION RATES

Typically, risk assessments conducted for the USDA Forest Service express application rates in units of lbs a.i./acre. These application rates are then used in the risk assessment to estimate exposures for workers (Section 3.2.2), members of the general public (Section 3.2.3), as well as various groups of non-target species (Section 4.2). Application rate in units of lbs a.i./acre is a particularly significant and in some respects a controlling parameter as input for environmental fate models to estimate concentrations in ambient water (Section 3.2.3.4). As noted in Section 2.3, several of the application methods used for imidacloprid – i.e., soil applications and tree injection – are not amenable to simple assessments of application rate in units of lbs a.i./acre and assumptions are needed in order to make such estimates.

For broadcast applications, on the other hand, application rates are typically expressed in units of lbs a.i./acre or can be readily converted to units of lbs a.i./acre. In assessing application rates, a distinction must be made between maximum amount that may be applied in a single applications and the maximum amount that may be applied in a single year. The product label for Provado 1.6 Flowable specifies a maximum single application rate per year for adelgid control on Christmas trees (N.O.S.), 40 fl oz/acre or 0.5 lb a.i./acre. The recommended application rates for adelgid control in any single application is 4 fl oz/acre to 8 fl oz/acre, corresponding to 0.05 lb a.i./acre to 0.1 lb a.i./acre. Thus, a total of 5 to 10 applications could be made in a given year.

The maximum application interval is specified as 7 days. No minimum application interval is specified.

All of the other formulations of imidacloprid that give application rates in terms of amount per acre have single maximum rates that are somewhat less than the specified maximum annual application rate of 0.5 lb a.i. for Provado 1.6 Flowable. For all of the other formulations used in broadcast applications (i.e., Marathon and Merit formulations), the maximum annual application rate is 0.4 lb a.i./acre.

Broadcast application rates for Marathon 1% are 15 oz formulation/1000 sq ft. This corresponds to 0.15 oz a.i./1000 sq ft, which in turn corresponds to about 0.009375 lb a.i./1000 sq ft [16 oz = 1 lb, 0.15 oz/16 oz/lb = 0.009375 lb]. Given than an acre corresponds to 43,560 sq ft, the application rate of 0.009375 lb a.i./1000 sq ft is equivalent to an application rate of 0.408 lb a.i./acre [0.009375 lb a.i./1000 sq ft x 43,560 sq ft/acre].

For Marathon II, broadcast application rates are given as 19.2 to 25.6 liquid oz/acre. These rates correspond to 0.15 gallons formulation/acre to 0.2 formulation gallons/acre [128 liquid oz = 1 gallon]. As noted in Table 2-2, Marathon II contains 2 pounds a.i./gallon. Thus, the application rates of 0.15 gallons formulation/acre to 0.2 gallons formulation/acre correspond to application rates of 0.3 lb a.i./acre to 0.4 a.i./acre.

Marathon 60 WP specifies a broadcast application rate of 1 packet per 3000 sq ft. Each packet contains 20 g of formulation corresponding to 12 g a.i. – i.e., 60% a.i. in the formulation. Twelve grams is equivalent to about 0.0265 lb a.i.[453.6 grams per pound, 12/453.6 = 0.026455...]. This corresponds to an application rate of about 0.385 lb a.i./acre [0.0265 lb a.i./3000 sq ft x 43,560 sq ft/acre].

Merit 75 WP specifies an application rate of 1.2 to 5.6 g formulation/1000 sq ft. This corresponds 0.9 g a.i./1000 sq ft to 4.2 g a.i./1000 sq ft – i.e., 75% a.i. in the formulation. The range of 0.9 g a.i. to 4.2 g a.i. is equivalent to about 0.00198 lb a.i. to 0.00926 lb a.i. and these amounts per 1000 sq ft would correspond to application rates of 0.086 lb a.i./acre [0.00198 lb a.i. x 43.56] to about 0.4 lb a.i./acre [0.00926 lb a.i. x 43.56 = 0.4034...].

Because the application of imidacloprid is very labor intensive, the Forest Service will limit applications to 1 per year. For this risk assessment, a single broadcast application scenario, ground or aerial, will be modeled an application of 0.4 lb a.i./acre. The single application of 0.4 lb a.i./acre is consistent with the highest single broadcast application rate for any of the imidacloprid formulations (Cowles 2005).

While these application rates will encompass the broadcast use of imidacloprid, they may not well represent exposures associated with soil injection, an application method that is highly relevant to forestry applications. Because soil injection involves placement of imidacloprid well below the soil surface, runoff and sediment losses, which are common mechanisms of offsite transport for soil surface or foliar applications, will be minimal in soil injection applications (Section 3.2). Conversely, but for the same reason, transport due to percolation is likely to be

higher in soil injection applications. In other words, the lack of significant runoff and sediment losses would tend to increase losses due to percolation because more of the chemical will be available for percolation. Target soil concentrations for soil injection applications could be used to model the potential for soil loss but target soil concentrations are not specified on any product labels for soil injection. For the current risk assessment, soil injection is modeled by setting the average soil incorporation depth to six inches.

In broadcast applications, mixing volumes of about 5 gallons per acre are recommended for aerial applications and 20 gallons per acre are recommended for ground applications of Provado 1.6 Flowable. The extent to which these formulations are diluted prior to application primarily influences dermal and direct spray scenarios, both of which are dependent on the 'field dilution' (i.e., the concentration of the pesticide in the applied spray). The higher the concentration of the pesticide in the field solution, the greater the risk. For this risk assessment, the lowest dilution will be taken at 5 gallons/acre, the minimum recommended for aerial applications. The highest dilution (i.e., that which results in the lowest risk) will be based on 20 gallons of water per acre, the application volume recommended for ground broadcast applications. The central estimate of the dilution rate will be taken as 10 gallons of water per acre. The exposures for applications of granular formulations are addressed as a special case as detailed in Section 3.2.2.

2.5. USE STATISTICS

The USDA Forest Service tracks and reports the use of pesticides on national forests by geographical areas referred to as "*Regions*". The Forest Service classification divides the U.S. into nine regions designated from Region 1 (Northern) to Region 10 (Alaska). The Forest Service then publishes the use statistics for pesticide applications to National Forests at http://www.fs.fed.us/foresthealth/pesticide/ reports.shtml. Currently (as of May 12, 2005), use statistics are given for the years 1998 to 2003. Based on these reports, only three applications of imidacloprid are reported, all of which occurred in Region 5 in a single forest (designated as Forest 3). Each application is reported to consist of 0.01 lb. The applications reportedly involved 41 seedlings (presumably a nursery application), 2.5 square feet, and 14.4 square feet. If these reports are accurate, all of these applications probably involved research projects and are not representative of the wider use of imidacloprid in forestry applications.

Currently, the maximum number of acres treated under projects on both Federal lands and Cooperative Suppression projects with states, is not anticipated to exceed 5,000 acres (2000 lbs. a.i.) annually. The most likely treatment area would be in the range of 1000 acres to 2000 acres (Onken 2005).

Imidacloprid is used on a number of crops and a summary of the agricultural uses of imidacloprid is presented in Figure 2-1 (USGS 1998a). These use statistics are for 1992, the most recent year for which data are available. As indicated in this figure, about 61,000 lbs of imidacloprid were applied to cotton (about 57% of total) and potatoes (about 43% of total). The geographic distribution of the agricultural uses of imidacloprid overlap but do not seem to be identical to the likely areas of forestry use (Regions 8 and 9). In Region 9 (the Northeast), agricultural use of imidacloprid is substantial and it does not appear to be likely that forestry uses will contribute significantly to the overall use of imidacloprid in Region 9. Relatively little

imidacloprid appears to be used in agriculture in the Southeast Region (Region 8) except in the northern part of Mississippi. Thus, in most areas of the southeast, the use of imidacloprid in forestry applications could be a substantial source of environmental levels of imidacloprid relative to agricultural use.

3. HUMAN HEALTH RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION

3.1.1. Overview

Imidacloprid is a neonicotinoid insecticide which produces neurotoxicity through binding or partial binding to specific areas of the nicotinic acetylcholine receptor. Acetylcholine is an important neurotransmitter in both insects and mammals; it is released at the nerve synapse in response to a membrane depolarization which is the hallmark of nerve transmission. There are different types of acetylcholine receptor. One type of receptor is called the nicotinic acetylcholine receptor (nAChR), which is activated by nicotine. Nicotine binds at or near the location where acetylcholine binds, causing the cascade of events leading to nerve transmission. Although imidacloprid activates nAChR, it is important to note that it does so in a manner fundamentally different from nicotine. This is important because unlike nicotine, imidacloprid is more toxic to insects than to mammals.

Imidacloprid and its nitrosoimine metabolite (WAK 3839) have been well studied in rats, mice and dogs. In mammals, the primary effects following acute high-dose oral exposure to imidacloprid are mortality, transient cholinergic effects (dizziness, apathy, locomotor effects, labored breathing) and transient growth retardation. Exposure to high doses may be associated with degenerative changes in the testes, thymus, bone marrow and pancreas. Cardiovascular and hematological effects have also been observed at higher doses. The primary effects of longer term, lower-dose exposure to imidacloprid are on the liver, thyroid, and body weight (reduction). Low- to mid-dose oral exposures have been associated with reproductive toxicity, developmental retardation and neurobehavioral deficits in rats and rabbits. Imidacloprid is neither carcinogenic in laboratory animals nor mutagenic in standard laboratory assays.

The nitrosoimine metabolite (WAK3839), which is an impurity of technical-grade imidacloprid, does not appear to be produced *in vivo* except after long-term high-dose exposure. The nitrosoimine metabolite is not mutagenic, and is of equivalent or lower toxicity than that of imidacloprid on the basis of acute and subchronic toxicity.

A summary of the toxicity data available for commercial formulations of imidacloprid that may be used in Forest Service programs is shown in Table 3-1. Product material safety data sheets (MSDS) are the source of the information shown in Table 3-1. Some of the information corresponds directly with registrant-submitted studies discussed below and summarized in Appendix 1. Other information, such as rat oral LD_{50} values for Marathon 1% Granular or Provado 1.6 Flowable, for example, apparently are used by analogy to studies conducted for other formulations.

3.1.2. Mechanism of Action

The mechanism of action of imidacloprid has been extensively studied in insects and mammals (Tomizawa and Casida 2003, 2004). Imidacloprid is a neonicotinoid insecticide which produces neurotoxicity through binding or partial binding to specific sub-sites or protein subunits of the nicotinic acetylcholine receptor (nAChR), which in turn activates nAChR activity.

Acetylcholine is an important neurotransmitter in both insects and mammals; it is released at the nerve synapse in response to a membrane depolarization which is the hallmark of nerve transmission. The acetylcholine then binds to a protein receptor in the membrane of the nerve synapse, which then opens/alters an ion channel, which in turn causes changes in the fluxes of ions (sodium, potassium, calcium, chloride), ultimately perpetuating the nerve impulse. The acetylcholine is subsequently destroyed by acetylcholinesterase, and the membrane returns to its normal resting state.

There are different types of acetylcholine receptor. One type of receptor is called the nicotinic acetylcholine receptor (nAChR), which is activated by nicotine. Nicotine binds at or near the location where acetylcholine binds, causing the cascade of events leading to nerve transmission. Nicotine and other substances which stimulate acetylcholine-like behavior through binding to nAChRs are called nAChR agonists. Imidacloprid is an nAChR agonist. It mimics the action of nicotine in the nervous system, binding at or near the site on the nAChR where nicotine binds, producing an unregulated barrage of nerve impulses, resulting in something akin to a nervous breakdown, and ultimately, death (Tomizawa and Casida 2003, 2004). Although imidacloprid activates nAChRs, it is important to note that it does so in a manner fundamentally different from nicotine. This is important because unlike nicotine, imidacloprid is more toxic to insects than to mammals.

In studies designed to investigate imidacloprid's selective toxicity, early investigators observed that radio-labeled imidacloprid binds to membranes of the head and brain in certain insects (e.g., house flies, cockroach, honey bee, cricket) but not to brain membranes of humans, dogs, mice, or chickens, suggesting that imidacloprid receptors are distributed differently in insects relative to mammals (Liu and Casida1993). Subsequent investigators determined that there are fundamental differences in the protein structure of nAChRs in mammals relative to insects (Buckingham et al. 1997; Chao et al. 1997; Liu and Casida 1993; Nagata et al. 1997, 1998; Matsuda et al. 2000; Nishiwaki et al. 2003; Tomizawa et al. 2001; Tomizawa and Casida 2003, 2004). Both imidacloprid and some of its metabolites show selective binding to nAChRs, with different affinities, depending on the structure of the metabolite and the nAChR subtype (Chao and Casida 1997; Yamamoto et al. 1998; Tomizawa et al. 2000, 2001; Tomizawa and Casida 1999, 2000, 2001; Shimomura et al. 2002, 2003, 2004; Zhang et al. 2002). In general, imidacloprid analogs or metabolites which bind with high affinity to insect nAChR, do so with low affinity to mammalian nAChR .

There is a correlation between the toxicity of imidacloprid/imidacloprid metabolites and the binding of a number of imidacloprid/imidacloprid metabolites to nAChR sub-sites (i.e., low toxicity and low-affinity binding in mammals, versus high toxicity and high-affinity binding in insects) (Tomizawa and Casida 2003, 2004). Taken together, the studies conducted with imidacloprid and its metabolites suggest that the guanidine or desnitro- metabolites may be activators of toxicity in mammals and detoxification products in insects, while the reverse is true for the nitrosoimine and olefin metabolites (Schulz-Jander and Casida 2002; Schulz-Jander et al. 2002). Desnitro-imidacloprid was more toxic (lower i.p. LD_{50}) in mice and showed greater affinity for nAChR (lower IC_{50}) in mouse brain than imidacloprid (Chao and Casida1997). However, in spite of high-affinity binding to nAChR in excess of the binding exhibited by

imidacloprid, the olefin metabolite was of low toxicity, probably due to detoxification. Desnitro-imidacloprid has been detected in kidney and liver tissues in rodents following imidacloprid exposure, which supports the idea that it is a toxic metabolite in mammals; brain tissue was not assessed for its presence (See Section 3.1.3.1 metabolism studies by Klein et al.). It is of note that the nitrosoimine metabolite (WAK 3839), which is of interest because it is a contaminant of technical grade imidacloprid and found in small quantities in food commodities, was not tested for binding affinity in the cited studies.

3.1.3. Pharmacokinetics and Metabolism

3.1.3.1. Metabolism – The metabolism of neonicotinoid compounds, including imidacloprid, is complex, and has been studied in plant crops, rodents, goats and laying hens (Tomizawa and Casida 2004).

Results from studies with rats and mice (Klein 1987a; Klein 1990; Klein and Karl 1990; Klein and Brauner 1991) indicate that there are two major routes by which the imidacloprid molecule is metabolized. Imidacloprid is a nitroguanidine molecule, composed of a pyridinyl moiety (a 6-member nitrogen-containing ring with a chloride substituent) and an imidazolidine ring (a 5-member ring with 2 nitrogens, with the $=N\text{-}NO_2$ nitroimine substituent on the carbon between the nitrogens). The first and predominant metabolic pathway involves oxidative cleavage which frees the pyridinyl moiety as 6- chloronicotinic acid. 6-Chloronicotinic acid is then either conjugated with glycine to form hippuric acid-type metabolites (major pathway), or de-chlorinated to form methylmercaptonicotinic acid and derivatives (minor pathway). The second biodegradation pathway entails hydroxylation of the imidazolidine ring to form 4- or 5- hydroxy imidacloprid. The hydroxylated compound may lose water to form the olefin metabolite. Studies with rats and mice suggest that the metabolism of imidacloprid does not vary with route of administration, sex of animal, or frequency of administration at low doses (1 mg/kg body weight) and acute or sub-acute exposures (1 to 14 days). However, at higher doses (20 mg/kg body weight), males appear to metabolize the parent compound more rapidly than females (Klein and Karl 1990).

In rats exposed orally or intravenously to ^{14}C-methylene labeled imidacloprid (Klein and Karl 1990), approximately 80% of the administered radioactivity was excreted in the urine, and approximately 72 % of the urinary radioactivity was identified: the primary metabolite 6-chloronicotinic acid and its glycine conjugate (WAK 3583) (approximately 28% of the total identified radioactivity), the olefin and hydroxy metabolites (NTN 35884, WAK 4103, and NTN 33823; approximately 30% of the identified radioactivity), and unchanged imidacloprid (approximately 15% of the identified radioactivity). About 11% of the radioactivity was recovered in the feces; of that, 7% was identified: 5% as hydroxy and olefin metabolites ;and 2% as unchanged imidacloprid. 6-Chloronicotinic acid and its glycine conjugate were not identified in the feces.

In rats exposed orally to ^{14}C-imidacloprid labeled at the 4- and 5- carbon of the imidazolidine ring (Klein and Brauner 1991), the following metabolites accounted for the radiation detected in the urine 48 hours after administration: KNO 0523 (19.1 - 34.7%: reduced imidazolidine moiety), NTN33968 (imine-substituted imidazolidine moiety: 8 - 18.4%), WAK4103 (5-

hydroxy-imidacloprid: 13.7 - 14.7%), NTN35884 (olefin metabolite: 7.7-9.1%) and imidacloprid (6.9 - 14.2%). Very little radioactivity was recovered in the feces, so the identity of fecal metabolites was not determined.

Imidacloprid residues were detected in the liver and kidneys of a lactating goat following repeated oral administration of a 10 mg/kg body weight dose of imidacloprid (Klein 1992). The study was designed to detect residues in edible tissue, and individual residues were identified only in liver and kidney tissues. The predominant residues in the liver were guanidine (NTN 38014) and a substituted chloropyridinyl moiety (WAK 4126). No unchanged imidacloprid was detected in the liver. The predominant metabolites detected in the kidneys were the olefinic metabolite (WAK 4103 and its glucuronide conjugate) and the glycine conjugate of 6-chloronicotinic acid (WAK 3583).

Studies with cytochrome p450 (CYP450) and flavin mono-oxygenase (FMO) isozymes from human liver were used to investigate the enzymatic basis for imidacloprid metabolism (Schulz-Jander et al. 2002; Schulz-Jander and Casida 2002). These studies demonstrate that CYP450, but not FMO isozymes, mediate imidacloprid metabolism. A single enzyme (CYP3A4) was identified as capable of mediating both oxidation at the imidazolidine moiety, and reduction of the imine substituent. 5-Hydroxy-imidacloprid (major) and olefin (minor) metabolites were produced by hydroxylation and de-saturation of the imidazolidine component, while nitrosoimine (major), guanidine (minor), and urea (trace) metabolites were produced by reduction and cleavage of the nitrosimine substituent.

The biokinetics and metabolism of imidacloprid and its nitrosoimine metabolite (WAK 3839) were studied in the rat (Klein 1990). Klein determined that the bio-kinetic behavior of WAK3839 and imidacloprid are similar; no significant differences in the absorption, distribution or excretion of radiation could be determined between these two compounds, when each was tested at a single low oral dose (1 mg/kg body weight). WAK 3839 was eliminated slightly more rapidly from the body than imidacloprid. However, the metabolism of WAK 3839 and imidacloprid were found to be quite different. The pattern of metabolite excretion following administration of imidacloprid was qualitatively and quantitatively similar to that described in previous studies. As in previous studies, WAK 3839 was not detected in either the urine or feces of rats given either a low (1 mg/kg body weight) or high oral dose (150 mg/kg body weight) of imidacloprid. However, rats given a low dose of WAK 3839 excreted primarily unchanged WAK 3839 in the urine, with only 8% of the excreted compounds attributable to NTN 33823 (a guanidine-type metabolite). These observations led the investigators to suggest that WAK 3839 is produced *in vivo* only after the pathways involved in the oxidative cleavage of imidacloprid to form 6-chloronicotinic acid are saturated (i.e., following long-term high-dose exposure). This hypothesis was supported in a subsequent study in which Klein (1990) fed rats and mice high doses of imidacloprid in the diet (2000 ppm) for one year. WAK 3839 was detected in the urine of both rats and mice under these conditions, at concentrations of 9 mg/100ml and 1.5 mg/100 ml, respectively.

3.1.3.2. Absorption – Human suicide case studies (Wu et al. 2001; Proenca et al. 2005)demonstrate that oral intake of imidacloprid formulations results in absorption and

distribution to the blood, kidneys, liver and lung (see Section 3.1.4 for details). Studies on animals suggest that imidacloprid is rapidly and completely absorbed following oral administration. After oral administration of ^{14}C-methylene labeled imidacloprid in rats, 95% of the administered dose was absorbed, with an estimated half-life of 35 minutes. The absorbed radioactivity was distributed rapidly throughout the body, with an approximate volume of distribution from the central compartment of 84% of the body volume. The maximum concentration of radioactivity was reached in the plasma within 2.5 hours. The kidney and liver had the highest concentrations of radiation, while the brain had the lowest concentrations. The distribution pattern of radioactivity throughout the body was independent of dose (Klein 1987b).

Similar results were obtained with ^{14}C-imidacloprid labeled at the 4- and 5- carbon of the imidazolidine ring (Klein and Brauner 1991). Following oral administration, greater than 90% of the administered radiation was estimated (from renal excretion data) to have been absorbed, with maximum concentrations reaching the plasma between 1 hour (1 mg/kg body weight dose) and 4 hours (150 mg/kg body weight). After 48 hours, the highest concentration of radioactivity was detected in the liver, with residual radiation in the total body at 1%. There were no differences in the pattern or distribution of radioactivity in comparison to the Klein (1987b) study.

In a separate study, Klein (1987a) used autoradiography to determine the distribution of ^{14}C-methylene labeled imidacloprid (NTN 33893) in male rats following oral and intravenous administration (20 mg/kg body weight). This study determined that imidacloprid distributes rapidly to all tissues with the exception of the fatty tissues, central nervous system and the mineral portion of bones, following either oral (1 hour) or intravenous (5 minutes) administration. With increased time following administration, radiation was also seen in the endocrine glands (thyroid, adrenals), the skin, and the walls of the aorta, indicating distribution and concentration of imidacloprid in these organs/tissues. Only small amounts of imidacloprid were found in the fatty tissues or central nervous system throughout the duration of the study. Concentrations decreased in most organs and tissues with increasing time following exposure. The pattern of distribution changed very little throughout the course of the study.

3.1.3.2.1. Dermal Absorption Rates – As detailed further in Section 3.2.2.2, two types of dermal exposure scenarios are considered in this risk assessment: those involving direct contact with a solution of the herbicide (e.g., immersion) and those associated with accidental spills of the herbicide onto the surface of the skin.

As detailed in SERA (2001), dermal exposure scenarios involving immersion or prolonged contact with chemical solutions use Fick's first law and require an estimate of the permeability coefficient, K_p, expressed in cm/hour. Using the method recommended by U.S. EPA (1992), the estimated dermal permeability coefficient for imidacloprid is 0.00013 cm/hour with a 95% confidence interval of 0.00007 - 0.00023 cm/hour. These estimates are used in all exposure assessments that are based on Fick's first law. For exposure scenarios like direct sprays or accidental spills, which involve deposition of the compound on the skin's surface, dermal absorption rates (proportion of the deposited dose per unit time) rather than dermal permeability rates are used in the exposure assessment. The estimated first-order dermal absorption

coefficient is 0.0015 hour^{-1} with 95% confidence intervals of 0.00067-0.0036 hour^{-1}. The calculations for these estimates are presented in Attachment 1. Note that the values for both dermal permeability and the first order dermal absorption rates are rounded to two significant figures in Table A1-5 of Attachment 1 and these values are entered into Worksheet A03 and used in all scenarios involving dermal exposures for both workers (Worksheet Series C) and the general public (Worksheet Series D).

3.1.3.3. Excretion – Studies with mammals suggest that imidacloprid is rapidly and completely eliminated in the urine and feces. Following oral or intravenous administration of ^{14}C-methylene labeled imidacloprid in rats (Klein 1987b), imidacloprid was rapidly absorbed and distributed throughout the body. The elimination of radioactivity from the plasma was described by two exponential components, with half-lives of 3 hours and 26-118 hours. More than 90% of the radioactivity was eliminated in the urine and feces in the first 24 hours following exposure. Approximately 96% of the administered dose was eliminated, of which 75% was found in the urine and 21% in the feces, within 48 hours of exposure. Less than 0.5% and 0.06% of the residual radioactivity were detected in the carcass and gastrointestinal tract, respectively (Klein 1987b).

The results of a metabolism study conducted by Klein and Karl (1990) agree well with the above results. In the Klein and Karl (1990) study, 90-98% of the administered radioactivity was recovered in the urine and feces of rats within 24 hours of administration. Approximately 78% of the recovered radioactivity in the urine and feces was identified. This finding was independent of the route of administration (oral versus intravenous), dose (1 mg/kg body weight versus 20 mg/kg body weight), or frequency of administration (single or repeated 14-day administration). Less than one percent of the administered radioactivity was recovered in the carcass.

In the Klein and Karl (1990) study, female rats exposed to high doses (20 mg/kg bw) excreted more radioactivity in the urine (79.5%) than similarly dosed males (73.3%) (Klein, 1990). These results are similar to those of Klein (1987a). Males on the other hand, excreted more in the feces (21.25%) than females (17.14%). Rats exposed to a single low oral dose of imidacloprid excreted similar amounts of radioactivity in the urine, and in the urine and feces, combined, in comparison to rats given the same dose via intravenous injection. However, rats given a single oral low dose excreted more in the feces than rats given the same dose via intravenous injection, suggesting the existence of a first-pass hepatic portal excretion at low doses. This effect disappeared when high dose oral exposure was compared with low-dose intravenous exposure.

Results of a study in rats (Klein and Brauner 1991) using ^{14}C-imidacloprid labeled at the 4- and 5- carbon of the imidazolidine ring were in agreement with the previously cited studies, with approximately 90% of the administered radiation excreted in the urine within 48 hours. However, unlike the studies conducted with ^{14}C-methylene labeled imidacloprid, very little radioactivity was recovered in the feces.

Heukamp (1992a) conducted a residue study with dairy cows to determine the status of imidacloprid and its olefin, hydroxy, 6-chloronicotinic acid and guanidine metabolites in milk

and edible tissues. Cows were given technical-grade imidacloprid in bolus capsules as follows: 0, 5 (1 dose), 15 (3 doses) or 50 (10 doses) mg NTN 33893 (97.6% a.i.)/kg feed. Total residues were detected as follows:

> Milk: Residues were not detected in the milk of controls or in cows given 1x 5 ppm dose on days 0, 1, 13 or 28 after exposure (0.02 ppm detection limit). Residues reached a plateau of 0.04 ppm and 0.14 ppm at doses of 3 x 15 and 10 x 50 ppm directly after the first exposure. Residues decreased with time.
> Muscle: Residues below detection (<0.02 ppm) in 1x 5 ppm cows; 0.03 ppm in 3 x 15 ppm cows and 0.12 ppm in 10 x 50 ppm cows.
> Fat: Residues (0.06 ppm) were detected only in 10 x 50 ppm cows.
> Liver: Residues were found at 0.05, 0.13 and 0.49 ppm from lowest to highest dose cows.
> Kidneys: Residues were found at 0.03, 0.1 and 0.3 ppm from lowest to highest dose cows.

In a study with a lactating goat, Klein (1992) determined that very little radiation following an orally administered dose of imidacloprid (nominal dose of 10 mg/kg body weight, daily, for three consecutive days, with ^{14}C-methylene labeled imidacloprid given on the final day) was detected in the milk (0.4% of the administered dose) 2 hours after the last administration, when plasma concentrations reached peak values. Of the administered dose, 46% was eliminated in the urine and 0.4% was excreted in the feces, 17% was detected in the liver, 14% was detected in the kidneys, 3.65% was detected in muscle, and 1.07% was detected in composite fat samples.

3.1.4. Acute Oral Toxicity

Wu et al (2001) reported a case of attempted suicide, in which an adult human male ingested 100 ml of an insecticide containing 9.7% imidacloprid with less than 2% of a non-specified surfactant and approximately 88% N-methyl pyrrolidone. Symptoms included sedation, dizziness, hemorrhagic gastritis, productive cough, fever, leukocytosis and hyperglycemia. The man recovered four days after the incident following aggressive medical intervention. It is important to note that most of the symptoms in this case were attributed to N-methyl pyrrolidone, as it was the main component in the insecticide formulation.

Proenca et al. (2005) report two human fatalities where suicide is attributed to imidacloprid. In the first case, a 33-year-old male was found dead by his wife. The initial autopsy was negative, except for a strange and intense smell. Subsequent pathology revealed only severe autolysis. Eventually, toxicological analysis revealed the presence of ethanol in the blood (0.018 g/L), as well as the presence of imidacloprid in the blood (12.5 ug/ml), kidney (13.6 ug/ml), liver (9.9 ug/ml), lung (20.6 ug/ml), and stomach contents (70 mg in 200 ml). In the second case, an empty bottle of Confidor® was found in association with a 66-year old male who had obviously committed suicide. In this case, no ethanol was found in body tissues, but imidacloprid was identified in the blood (2.05 ug/ml), urine (0.29 ug/ml), kidney (2.5 ug/ml), liver (1.01 ug/ml), lung (8.8 ug/ml) and stomach contents (37.1 mg in 150 ml). In the second case, the autopsy revealed signs of chemical burns in the gastrointestinal tract, as well as pulmonary edema and a yellow liver. Histopathological findings from poorly preserved samples indicated signs of right-sided cardiac insufficiency and revealed dark granular spots in the lungs.

Studies conducted with animals to address the acute oral (gavage) toxicity of imidacloprid, imidacloprid formulations and the nitrosoimine metabolite of imidacloprid are summarized in detail in Appendix 1. Several acute intraperitoneal injection studies are also summarized in Appendix 1, and support the findings of the gavage studies. The majority of these studies were submitted in response to EPA's requirements as part of the pesticide registration process. Several of the studies conducted with mice and hamsters were completed to fulfill genotoxicity testing requirements, but are also included in Appendix 1 because they address mortality and clinical signs of toxicity.

On the basis of acute mortality, these studies suggest that technical grade imidacloprid is more toxic than imidacloprid formulations, and more toxic than its nitrosoimine metabolite (not the des-nitro metabolite). The lowest LD_{50} value for technical grade imidacloprid, 131 mg/kg body weight, was detected in male mice (Bomann et al. 1989b). The lowest LD_{50} value for the nitrosoimine metabolite (NTN 37571 or WAK 3839), 200 mg/kg, was detected in fasted male or female mice (Nakazato 1988a). On the basis of the observed LD_{50} values, imidacloprid and its nitrosoimine metabolite are classified by EPA as slightly to moderately toxic.

In general, experimental animals showed signs of toxicity at doses lower than those causing mortality, regardless of the species, formulation or metabolite administered. In most studies, clinical signs of toxicity, including staggering gait, sedation, apathy, tremors, labored breathing and convulsions (higher doses) were observed shortly after dosing; these signs were typically resolved in all animals prior to the end of a study (day 14). Transient decrease in body weight was also a common symptom of imidacloprid-treated animals.

An acute oral neurotoxicity screening study conducted by Sheets (1994a, b) is of particular importance, given imidacloprid's mechanism of action. In this study, there were decreased measures of motor and locomotor activity in females at doses of 42 mg technical grade imidacloprid/kg body weight and higher. These signs, which resolved within 7 days, were attributed to acute cholinergic toxicity. There were no effects in females at a dose of 20 mg/kg body weight (NOAEL). EPA used the LOAEL of 42 mg/kg body weight as the basis for the acute RfD for imidacloprid. Dividing the LOAEL of 42 mg/kg by an uncertainty factor of 300 (10 for interspecies extrapolation, 10 for intraspecies sensitivity, 3 for using an LOAEL to approximate an NOAEL), EPA derived an acute RfD of 0.14 mg/kg (U.S. EPA/OPP 2003).

For those who questioned (i.e., the National Resources Defense Council: NRDC) why EPA did not use the NOAEL as the basis for the RfD, U.S. EPA responded with the following clarification:

> *In its objections to a separate imidacloprid tolerance action, NRDC claims that EPA erred by regulating on the basis of a LOAEL for acute and chronic toxicity. As can be seen from the above table, NRDC is mistaken with regard to use of a LOAEL for estimating the RfD for chronic risk. The acute toxicity endpoint was based upon a LOAEL of 42 mg/kg/day from an acute neurotoxicity study in rats. This value was adjusted with a safety factor of 3X to approximate the value of a NOAEL. EPA has high confidence that this value of 3x is sufficient for several reasons. The effect seen at the LOAEL in the acute neurotoxicity study (decreased motor activity), occurred only in one sex of the rat (females), was characterized as minimal, and may have been a result of the use*

of the gavage dosing in the study. The decreased motor activity was not replicated following repeated dietary administration (non-gavage) at lower and higher doses (10, 70 or 200 mg/kg/day) in the subchronic neurotoxicity study in the same species (rats). Further, using a safety factor of 3X produces a regulatory endpoint lower than the acute effect levels in other standard studies for determining an acute endpoint, developmental toxicity studies in two species, and in another study that is on occasion used for such a purpose, the developmental neurotoxicity study in rats. – U.S. EPA/OPP 2003

Based on the available studies, it is not possible to draw an unequivocal conclusion on whether there are gender differences in acute toxicity. This is of interest, given that male rats showed a marginally higher rate of metabolism than females with exposure to imidacloprid at higher doses (Klein and Karl 1990).

3.1.5. Subchronic or Chronic Systemic Toxic Effects

Studies that investigate the subchronic and chronic systemic toxicity of imidacloprid in mammals are summarized in Appendix 2. With one exception, all of these studies involved dietary administration of technical grade imidacloprid, and all were conducted in response to EPA requirements for testing under the pesticide registration process. One study was conducted with the nitrosoimine metabolite WAK 3839, as discussed below.

Studies suggest that oral ingestion of imidacloprid can cause growth retardation and adverse effects on the liver, thyroid, testes, heart, thymus, bone marrow, pancreas and nervous system. Degenerative changes in the bone marrow, thymus and pancreas were reported only in dogs fed high doses (5000 ppm) of imidacloprid (Bloch 1987). Tubular degeneration of the testes was seen in both dogs and rats in subchronic range-finding studies where imidacloprid was administered at higher doses (3000 to 5000 ppm diet) (Bloch 1987; Eiben 1988). It is important to note that effects seen at the lowest doses of imidacloprid exposure in mammals ***were not*** on the nervous system. Nervous system effects are discussed in detail in the following section (3.1.6).

A key study which investigates the chronic toxicity and carcinogenicity of imidacloprid was conducted by Eiben and Kaliner (1991) and Eiben (1991) with rats. This study is the basis for EPA's Chronic RfD of 0.057 mg/kg/day for imidacloprid (EPA/ORD 2005). The critical effects seen in this study are depression in body weight gain (both sexes) and mineralization of the colloid of the thyroid follicles (both sexes, but males affected at a lower dose), yielding a NOAEL of 5.7 mg/kg body weight/day (100 ppm diet). The effect on the thyroid at lower doses in males is intriguing, given that males have been shown to metabolize imidacloprid more rapidly than females at doses in excess of 1 mg/kg/day (Klein and Karl 1990). This suggests, but does not prove, that a metabolite of imidacloprid may be the proximate cause of thyroid toxicity.

As discussed in Section 3.3.2, the chronic RfD derived by U.S. EPA/OPP (2003) is based on the studies by Eiben and Kaliner (1991) and Eiben (1991) in which mineralization of the colloid of the thyroid follicles was noted in male and female rats. Consequently, it is important to note that rats may be more sensitive than other species with regard to mineralization of the colloid of the thyroid follicles. Lewandowski et al. (2004) note that the thyroid follicles in rats are much smaller, and contain much less colloid than primates. Hence, the smaller colloid reserve could

lead to a greater susceptibility to effects on the thyroid in rats, than in primate species such as humans, which have a larger colloid reserve.

Most studies report retardation of growth expressed as reduced body weight gain. These changes tend to be reversible at the lower doses and shorter periods of imidacloprid administration (see acute toxicity studies, Appendix 1), but are more pronounced and irreversible at higher doses or following longer periods of administration (Bloch, 1987; Eiben 1988a,b,1989, 1991; Watta-Gebert 1991a, b; Eiben and Kaliner 1991). It is not possible to attribute these changes to reduced food consumption because the evidence does not indicate a correlation between food consumption and body weight reduction (e.g., food consumption was reduced in some studies, but significantly increased in others). It is likely that the observed deficit in growth in some cases may be secondary to adverse treatment-related changes in the liver (increases or decreases in plasma cholesterol; altered glucose concentrations; enzyme induction and multi-focal group cell necrosis [higher doses]), to fundamental changes in metabolic rate (e.g., effects on the thyroid), and/or to degenerative changes in the tissues and organs related to the digestive system (e.g., degeneration of salivary glands).

It is also important to note that imidacloprid may have an adverse effect on the cardiovascular system. Klein et al. (1987a) found that imidacloprid distributes to the walls of the aorta. Eiben (1988) observed decreased absolute and relative heart weights in mice fed a high concentration of imidacloprid in the diet (3000 ppm) and observed an increased incidence of death (reported by this investigator as *heart attack*) during blood withdrawal. Watta-Gebert (1991a,b) also observed that male mice exposed to 2000 ppm imidacloprid in the diet died more frequently from *heart attack* (not otherwise specified) during manipulation (blood withdrawal, anesthesia, tattooing etc.) than controls.

One subchronic dietary study was conducted on rats with the nitrosoimine metabolite (WAK 3839) of imidacloprid (Krotlinger 1992). The results of this study are different than those observed following imidacloprid administration in any species, suggesting that the nitrosoimine metabolite is not responsible for the toxicity observed in studies conducted with imidacloprid. This is consistent with the observation that the nitrosoimine metabolite is not normally produced *in vivo* in rats and mice, following lower-dose consumption of imidacloprid, and is only found at relatively low concentrations following higher-dose (imidacloprid at 2000 ppm diet) and longer term (1 year) exposure (See Section 3.1.3.1; the metabolism study by Klein 1990).

3.1.6. Effects on Nervous System

Extensive studies on the mechanism of action of imidacloprid demonstrate that imidacloprid is a nicotinic acetylcholine receptor agonist (Section 3.1.2. Mechanism of Action). However, unlike nicotine, imidacloprid binds with lower affinity in mammals than it does in insects, producing lower acute toxicity in mammals than in insects.

Acute, subchronic and developmental studies have been conducted in mammals specifically to investigate the neurotoxicity of technical grade imidacloprid in response to EPA's pesticide registration requirements. These studies suggest that neurobehavioral and pathological effects are seen only after high dose exposure to imidacloprid. Acute oral toxicity studies (Appendix 1)

as well as comprehensive subchronic and chronic toxicity studies (Appendix 2) failed to find any treatment-related impacts on measured plasma or brain cholinesterase activities in mammals.

An acute oral neurotoxicity screening study was conducted by Sheets (1994a,b) with rats (see Appendix 1 for details). In that study, ingestion of imidacloprid at doses of 42 mg/kg body weight and higher were associated with symptoms of cholinergic toxicity (signs of motor and locomotor deficits such as sedation, apathy, staggering gait, trembling, and labored or accelerated breathing).

A thirteen-week neurotoxicity screening study (Appendix 2) found no evidence of motor/locomotor impairment in a series of tests conducted on rats fed up to 3027 ppm technical grade imidacloprid in the diet (Sheets and Hamilton 1994). There were no gross or microscopic lesions in the nerve or muscle tissue among these rats. However, deficits in the neurobehavioral functional observational battery were observed in males fed the highest dose (3027 ppm, equivalent to 196 mg imidacloprid/kg body weight/day). The NOAEL for neurobehavioral effects in this study is 69.1 mg/kg body weight/day (963 ppm).

A developmental neurotoxicity screening study was conducted with rats (Sheets 2001). This study is presented in detail in Appendix 2 in the "Teratology" subsection. Rats were fed 0, 100, 200, 250 or 750 ppm technical grade imidacloprid in the diet from gestation day 0 through lactation day 21. The only effect on maternal rats was a 14% reduction in food consumption at the highest dietary concentration. There were no effects on reproductive variables. Following an extensive battery of tests, the only neurological effect observed in the F1 offspring was reduced activity in the figure-eight maze on post-natal days 17 (both sexes) and 21 (females only) relative to controls, among rats whose mothers were exposed to the highest dose (750 ppm). There were no effects on the brain or histopathological changes in the brain, neural tissues or skeletal muscle. The NOAEL for neurological effects in this study is 250 ppm (equivalent to maternal doses of 19.4 - 19.7 mg/kg body weight/day during gestation; and 30.0 - 45.4 mg/kg body weight/day during lactation).

All of the above studies, conducted with rats, found no imidacloprid-related histopathological changes in the brain. However, in a supplementary 24-month carcinogenicity study conducted with mice, Watta-Gebert (1991b) observed an increased incidence of mineralization of the thalamus in the brains of mice fed 2000 ppm technical grade imidacloprid in the diet. This dietary concentration was equivalent to mean doses of 413.5 and 423.9 mg imidacloprid/kg body weight/day for males and females, respectively.

One human case study (Wu et al. 2001), and studies conducted on laboratory animals with low percentage imidacloprid formulations (See Appendix 1) suggest that effects such as dizziness and labored breathing may also be caused by ingredients in imidacloprid formulations other than imidacloprid (e.g., N-methyl pyrrolidone).

3.1.7. Effects on Immune System

With two exceptions, comprehensive acute (Appendix 1), subchronic, and chronic toxicity (Appendix 2) studies conducted with imidacloprid and imidacloprid formulations did not find

any effects which could be related to decrements in immune function or overstimulation of immune function in mammals. In a four- week inhalation study where rats were exposed to 191.2 mg technical grade imidacloprid dust/m3 air via inhalation, a slight depression in thymus weight was observed relative to controls (Pauluhn 1989). Decreased thymus weight relative to controls was also observed in a 28-day range-finding study conducted with dogs fed 5000 ppm (49.0 mg/kg body weight/day) imidacloprid in the diet (Bloch 1987). However, neither this effect, nor effects on the spleen, lymphocyte counts, or lymph tissue were observed in a study of dogs exposed to lower dietary concentrations (up to 1800 ppm) of imidacloprid (Ruf 1990), or in comprehensive chronic exposure studies with rats or mice, suggesting that imidacloprid does not have a primary effect on the immune system.

The nitrosoimine metabolite (WAK 3839) of imidacloprid does appear to have an effect on the immune system; it caused significantly increased lymphoctye counts and lower numbers of polymorphonuclear cells relative to controls in rats fed 110 ppm WAK 3839 in the diet (equivalent to a dose of 13 mg/kg body weight/day) for 12 weeks (Krotlinger 1992). The relevance of this finding with regard to direct imidacloprid use and exposure is uncertain, given that WAK 3839 has not been shown to be produced *in vivo* under likely conditions of exposure (i.e., low-dose exposure). However, it does suggest that high concentrations of the nitrosoimine metabolite resulting from the environmental degradation of imidacloprid could lead to potential immune system disruption.

3.1.8. Effects on Endocrine System

In autoradiographic and metabolic studies conducted with rats, Klein et al (1987a, b) determined that radiation from orally administered ^{14}C-methylene labeled imidacloprid appears rapidly in thyroid and adrenal tissues. No pathological findings involving adrenal tissues were reported in the comprehensive acute, subchronic and chronic exposure studies conducted on rats, mice, and dogs with imidacloprid and imidacloprid formulations. However, degenerative changes in the thyroid have been detected in dogs (follicular atrophy) fed 5000 ppm technical grade imidacloprid for 28 days (Bloch 1987); in rats (mineralization of colloid follicles) fed 300 or 900 ppm technical grade imidacloprid for 24 months (Eiben and Kaliner 1991) and in rats fed 1800 ppm technical grade imidacloprid for 24 months (Eiben 1991).

3.1.9. Teratogenic and Reproductive Effects

3.1.9. 1. Teratology Studies – Imidacloprid has been tested for its ability to cause birth defects (teratogenicity), developmental toxicity, and reproductive impairment in pregnant rabbits (Becker et al. 1992) and rats (Becker et al. 1992b) . These studies, summarized in Appendix 2, were conducted to fulfill EPA requirements for testing as part of the pesticide registration process. In summary, imidacloprid was not found to affect reproductive variables or cause birth defects at doses which did not cause maternal toxicity, when the pregnant animals were exposed via gavage during the critical developmental phases of their pregnancy. However, imidacloprid may adversely affect reproduction and cause developmental delays as a result of maternal toxicity. A developmental neurotoxicity screening study in rats (Sheets 2001) suggests that imidacloprid may also cause neurotoxicity in offspring born to imidacloprid-exposed mothers at doses which do not cause maternal toxicity.

In the Sheets (2001) study, pregnant rats were fed technical grade imidacloprid throughout pregnancy and lactation at doses of 0, 100, 250 and 750 ppm. No effects other than significantly reduced food consumption (14% relative to controls) was observed in the mother rats. However, decreased body weight gain and reduced activity in the figure-eight maze, relative to controls, were seen among the offspring of mothers fed the highest dose of imidacloprid (750 ppm; equivalent to 54.7 to 155.0 mg imidacloprid/kg body weight/day).

Skeletal abnormalities (non-ossified phalangeal nuclei and metacarpalia of the fore and hind limbs) and reduced body weights, relative to controls, were observed in rabbits born to imidacloprid-exposed (72 mg/kg body weight/day) mothers (Becker and Biedermann1992). The mother rabbits had reduced body weight gain during gestation, abortions, and total litter resorptions. The NOAEL for maternal toxicity was 8 mg/kg/day and the NOAEL for fetal and reproductive toxicity was 24 mg/kg/day.

Skeletal anomalies (wavy ribs) were observed in rats whose imidacloprid-exposed (100 mg/kg body weight/day) mothers had reduced body weight gain during gestation (Becker et al. 1992). Effects on reproductive variables or fetal body weights were not observed. The NOAEL for maternal toxicity (reduction in body weight gain relative to controls) was 10 mg/kg/day, the NOAEL for reproductive toxicity was 100 mg/kg/day, and the NOAEL for fetal toxicity (wavy ribs) was 30 mg/kg/day.

3.1.9. 2. Multigeneration Reproduction Studies – Imidacloprid was tested for its ability to adversely affect reproduction when multiple generations of rats were exposed in the diet (Suter et al. 1990). This study was conducted according to EPA guidelines for testing as part of the pesticide registration process, and is summarized in Appendix 2. Imidacloprid was not found to affect reproductive variables or cause birth defects. However, reduced mean body weight and body weight gain relative to controls was observed in the offspring of all generations at the highest dietary concentration tested (700 ppm). At this concentration, parental animals also had reduced body weights, relative to controls, in association with reduced food consumption.

3.1.9. 3. Target Organ Toxicity – Two subchronic studies conducted with technical grade imidacloprid suggest that repeated high-dose exposure may result in testicular degeneration in mammals. Tubular degeneration of the testes was observed in dogs fed 5000 ppm imidacloprid in the diet for 28 days (Bloch, 1987). "Low-grade degenerative changes" in testicular tubuli were reported in a study of rats fed 3000 ppm imidacloprid in the diet for 98 days (Eiben 1988a). These studies are summarized in Appendix 2.

3.1.10. Carcinogenicity and Mutagenicity

3.1.10.1. Bioassays for Carcinogenicity – There are no human or animal studies which suggest that imidacloprid causes cancer. Technical grade imidacloprid has been tested in comprehensive carcinogenicity studies with rats (Eiben and Kaliner 1991; Eiben 1991) and mice (Eiben 1988b; Watta-Gebert 1991a,b). These studies were conducted in accordance with EPA guidelines for testing, and are summarized in Appendix 2. No changes in time-to-tumor development or increases in the incidence of tumors among animals exposed to maximum tolerated doses of

imidacloprid throughout their lifetimes, relative to non-exposed controls, were found in any study. However, systemic toxic effects, as discussed in the previous sections, were observed.

Based on a lack of carcinogenic activity in animals, EPA classifies imidacloprid as Group E with respect to carcinogenicity (i.e., no evidence of carcinogenicity) (EPA/ORD 2003, 2005).

3.1.10.1. Mutagenicity – The available data indicate that neither imidacloprid nor its nitrosoimine metabolite, WAK 3839, are mutagenic or genotoxic (cause damage to DNA). One study suggests that imidacloprid and other pesticides may render an organism more susceptible to DNA damage; Shah et al.(1997) found that imidacloprid-exposed calf thymus cells had significantly more DNA adducts (indicative of DNA damage) than unexposed control cells. It is important to note that this study was conducted *in vitro*, and that *in vivo* studies cited in the following paragraphs failed to identify any imidacloprid-induced DNA damage.

Imidacloprid did not cause mutations, with or without metabolic activation, in the *Salmonella typhimurium* reverse mutation assay (Watanabe 1991; Herbold 1989a) or CHO-HGPRT forward mutation assay (Lehn 1989a). Imidacloprid showed weak clastogenic activity (DNA breakage) in the presence of metabolic activation in one of two trials with human lymphocytes (Herbold 1989c). However, *in vivo* studies with orally administered imidacloprid failed to demonstrate clastogenic effects. Negative results were obtained in Chinese hamster bone marrow (Herbold 1989b,d), a mouse micronucleus test (Herbold 1988a), and a mouse germ cell test (Volkner 1990).

Imidacloprid did not induce sister chromatid exchange in Chinese hamster ovary (CHO) cells at concentrations up to 1250 ug/ml with metabolic activation (Putnam and Morris 1989), but caused significant increase in sister chromatid exchange at concentrations where compound precipitation and cellular toxicity was evident (2 and 3 mg/ml, with metabolic activation) (Taalman 1988).

Imidacloprid did not cause DNA damage in bacterial spores (Watanabe 1990), stimulate unscheduled DNA synthesis in the primary hepatocytes of rats (Cifone 1988), or induce mitotic recombination in yeast (Herbold 1988b).

The nitrosoimine metabolite WAK 3839 did not cause DNA damage in the mouse micronucleus test following oral or intraperitoneal administration (Usami1988a,b; Herbold 1989e,f). WAK 3839 was similarly negative in tests for unscheduled DNA synthesis (Fautz 1989), reverse mutation in *Salmonella typhimurium* (Watanabe 1990), and chromosomal aberrations (Heidemann 1989) or forward mutations in the V79-HGPRT and CHO-HGRT assay systems (Lehn 1989b,c).

3.1.11. Irritation and Sensitization (Effects on the Skin and Eyes)

3.1.11.1. Skin Irritation – A number of standard assays for skin irritation have been conducted in response to EPA pesticide registration requirements, and are summarized in Appendix 1. These studies demonstrate that imidacloprid is not a skin irritant (Sheets 1990c,d,i; Pauluhn 1988c). However, some imidacloprid formulations are slight or mild irritants (Sheets and

Phillips 1991c; Wakefield 1996b; Warren 1995d; Robbins, 1996b), suggesting that some or all of the inert ingredients may be responsible for the observed irritation.

3.1.11.2. Skin Sensitization – Imidacloprid and imidacloprid formulations have been tested to see whether they cause allergic reactions when applied to the skin (i.e., dermal sensitization). These studies, summarized in Appendix 1, were conducted with guinea pigs, mice and rabbits, in accordance with EPA guidance in the pesticide registration process. Based on these studies, neither imidacloprid nor its formulations cause dermal sensitization (Sheets 1990e; Ohta 1988; Sheets 1990j; Sheets and Phillips 1991d; Warren 1995e; Pritchard and Donald 2004e).

3.1.11.3. Ocular Effects – Studies conducted with rabbits in accordance with EPA test guidelines for pesticide registration demonstrate that imidacloprid is not an eye irritant (Pauluhn 1988b). However, some imidacloprid formulations are mild to moderate eye irritants (Sheets 1990c,h; Astroff 1992; Sheets and Phillips 1990, 1991; Astroff and Phillips 1992; Warren 1995c; Robbins 1996a), indicating that components other than imidacloprid are responsible for the observed irritation. These studies are summarized in Appendix 1.

3.1.12. Systemic Toxic Effects from Dermal Exposure

The assessment of dermal toxicity of imidacloprid in experimental animals is important, since dermal exposure is most likely in people who use imidacloprid formulations. Studies (Appendix 1 and 2) of dermal toxicity submitted to EPA in response to pesticide registration requirements show that neither technical grade imidacloprid nor its various formulations produce mortality or toxicity through dermal exposure. Publically available information from Bayer (http://www.animalhealth.bayer healthcare.com) states that the canine/feline topical imidacloprid anti-flea treatment (Advantage® contains 10% imidacloprid as a.i.) remains localized in the superficial lipid layer of the skin and is not appreciably absorbed. Bayer scientists reported that fleas were not killed when exposed to dogs previously treated with imidacloprid but whose skin and fur had been cleansed of all "active material", which suggests that systemic distribution of imidacloprid is not relevant to its efficacy against fleas following dermal application.

3.1.13. Inhalation Exposure

An inhalation exposure study with rats conducted with technical grade imidacloprid yielded LC_{50} values in excess of the highest experimental concentration (5223 mg imidacloprid/m^3) (Pauluhn 1988a,d). As with oral exposure studies, the effects related to exposure were marginally reduced body weight gain and some transient clinical signs, including difficult breathing, reduced mobility and slight tremors. Similar results were obtained in studies with most other imidacloprid formulations similarly tested (Warren 1990a,b,c; Warren 1991; Warren and Berry 1995). One exception is a formulation designated NTN 33893 75% WP-WS, which yielded an acute LC_{50} value of 2650 mg formulation/m^3 in male rats. There were also transient but significant reductions in body weight gain, and transient clinical signs including ataxia, convulsions, hypo-activity and tremors. These results suggest that some of the inert ingredients in the 75% WP-WS formulation may either independently produce adverse effects or potentiate the effects produced by imidacloprid.

Short-term multiple inhalation exposure studies conducted with rats (Pauluhn 1988a,d, 1989) yielded results similar to those observed in oral exposure studies, with one additional symptom. Imidacloprid-exposed rats in the Pauluhn studies had significantly reduced blood clotting times and increased urine pH relative to air-only exposed controls. The investigators stated that these changes were related to functional changes in the liver (induction of hepatic mixed function oxidases was the most sensitive endpoint in these studies), although neither of these conditions were observed in orally exposed rats whose livers were also adversely affected by imidacloprid exposure. The NOAEC for inhalation exposure from these studies is 5.5 mg imidacloprid/m^3 air.

3.1.14. Inerts and Adjuvants

Because the nature of the inert components of imidacloprid formulations is proprietary, there isn't much publically available information. Crystalline quartz silica and napthalene have been identified as inert ingredients in Merit 0.5 and Leverage 2.7, respectively (Cox 2001). One human case study, in which a man attempted suicide by ingesting an imidacloprid-containing insecticide (Wu et al. 2001), reported that the formulation contained 10% imidacloprid, less-than 2% inerts, and the balance composed of N-methyl-pyrrolidone solvent. Shiotsuka (1991) reported that chemically distinct forms of bentonite are solid inerts in the 0.62 and 2.5% granular formulations. A summary of product characteristics available on the internet lists butylated hydroxytoluene as an antioxidant in the imidacloprid-containing flea product "Advantage 250 for Dogs". A complete list of known inerts contained in commercial formulations of imidacloprid that may be used in Forest Service Programs for the control of Adelgid species is shown in Table 2-3.

The results of acute oral toxicity studies conducted on laboratory animals with imidacloprid and various imidacloprid formulations suggest that none of the inert components in the formulation are more toxic or potentiate greater toxicity than imidacloprid alone (i.e. the lowest LD_{50} and NOAEL values were from studies conducted with technical grade imidacloprid), when exposure is short-term and oral. However, inhalation of NTN 33893 75% WP-WS led to greater mortality and toxicity than inhalation of technical grade imidacloprid (Warren 1991), suggesting that the inert components of this formulation may potentiate imidacloprid toxicity. In addition, certain imidacloprid formulations produced mild to moderate eye and skin irritation (BAY NTN 33893 2.5% Granular, BAY NTN 33893 0.5% Granular, BAY NTN 33893 0.62% Granular, BAY NTN 33893 75% WP-WS, BAY NTN 33893 240 F.S., BAY T-7391 10% Pour-On and Pointer Insecticide), while technical grade imidacloprid did not.

3.1.15. Impurities and Metabolites

An overview of the toxicology of imidacloprid and its nitrosoimine metabolite, WAK 3839, was provided to EPA by the registrant as part of the pesticide registration process. In this submission, Sangha and Machemer (1992) state that the technical grade imidacloprid used in toxicology studies contained, on average, 30 ppm of WAK 3839.

The metabolites produced through *in vivo* metabolism and environmental degradation processes are numerous and well known, and have been discussed in other sections of this document. Since all of the *in vivo* toxicology studies on imidacloprid involve the generation of metabolites, the potential toxicity of the metabolites should be encompassed by the available toxicity data.

WAK 3839 has been shown to be produced *in vivo* only following long-term high dose oral exposure (Klein 1990), and then, only in low quantities. Pharmacokinetic (Klein 1990), acute (Nakazato 1988b, 1990; Ohta 1991), and subchronic (Krotlinger 1992) oral toxicity tests suggest that WAK 3839 acts differently in the body than imidacloprid, producing different toxic effects. Rats exposed to 300 or 900 ppm of the nitrosoimine metabolite in the diet for 12 weeks had changes in white blood cells, which indicate potential immune system disruption. WAK 3839 is less acutely toxic than technical grade imidacloprid in rats and mice, and was not shown to be mutagenic or genotoxic (Usami1988a,b; Herbold 1989e,f; Fautz 1989; Watanabe 1990b; Heidemann 1989; Lehn 1989b,c).

3.1.16. Toxicologic Interactions

No information on potential toxicologic interactions between imidacloprid and other chemicals in mammalian species was located in the available literature. However, imidacloprid may interact with other chemicals that cause liver damage, as imidacloprid has been shown to cause liver damage, and to induce liver enzymes such as cytochrome P-450.

In experiments with insects, piperonyl butoxide, an inhibitor of P-450 monooxygenases, has been shown to have a synergistic effect on the acute toxicity of imidacloprid (Zewen et al. 2003). Along with other studies on cat fleas, house fly, tobacco whitefly and green peach aphid, cited by Zewen et al (2003), this suggests that P-450 monooxygenases play an important role in the detoxification of imidacloprid and the development of imidacloprid resistance in insects.

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview

As discussed in Section 2.3.4, the exposure assessments for this risk assessment are detailed in four sets of worksheets:

broadcast applications of liquid formulations on clay or loam soils;
broadcast applications of granular formulations on clay or loam soils;
soil injections in clay or loam soils;
applications (any method) to predominantly sand soils.

No quantitative exposure assessments are given for tree injection of imidacloprid; this application method is extremely specific to the targeted species (adelgids) and the plant to be protected (hemlocks). There is no apparent basis for asserting that human exposures due to tree injection are likely to be substantial, and there are no methods and no information sufficient to quantify the exposures except to suggest that the exposures will be less than those associated with other application methods. A similar problem exists for workers applying imidacloprid by soil injection. While it seems plausible that soil injection applications will lead to exposures that are less than those associated with more standard broadcast applications, very little information is available to substantiate this supposition. Thus, for workers involved in soil injection application, the exposure assessment is based on exposure rates associated with backpack applications. These will almost certainly overestimate worker exposures during soil injection and these overestimates may be extreme.

For both workers and the general public, exposure assessments are presented for both aerial and ground broadcast applications. These applications are included at the request of the Forest Service in response to comments from cooperators (other local, state, or federal governmental organizations) who may wish to consider these application methods. In Forest Service programs, however, only tree injection and soil injection applications are anticipated.

Central estimates of exposure for workers are approximately 0.005 mg/kg/day for aerial and backpack workers and about 0.009 mg/kg/day for broadcast ground spray workers. Upper ranges of exposures are approximately 0.06 mg/kg/day for backpack and aerial workers and about 0.03 mg/kg/day for broadcast ground spray workers. All of the accidental exposure scenarios for workers involve dermal exposures and these accidental exposures lead to estimates of dose that are comparable to or substantially below the general exposure estimates for workers.

For the general public, the range for acute exposures is about 0.00000001 mg/kg bw to about 0.3 mg/kg bw. For soil injection applications, all non-accidental exposures are extremely low. For all application methods, the upper range of exposure is associated with scenarios involving the accidental spill of imidacloprid into a relatively small body of water.

For chronic (long-term) exposures, the modeled exposures are much lower than for acute (short-term) exposures. The highest chronic exposure is about 0.09 mg/kg/day and is associated with the consumption of contaminated broadleaf vegetation after broadcast applications of liquid formulations. For soil injection, the method that may be used in Forest Service programs, the

highest chronic exposure is 0.000001 mg/kg/day and is associated with the consumption of contaminated water after application to sandy soil. As noted in the program description, the Forest Service does not anticipate applying imidacloprid to sandy soils and the corresponding exposures associated with clay or loam soils are negligible.

3.2.2. Workers

The Forest Service uses a standard set of exposure assessments in all risk assessment documents. While these exposure assessments vary depending on the characteristics of the specific chemical as well as the relevant data on the specific chemical, the organization and assumptions used in the exposure assessments are standard and consistent. All of the exposure assessments for workers as well as members of the general public are detailed in the worksheets that accompany this risk assessment. Detailed documentation for these worksheets is presented in SERA (SERA 2004a). This section on workers and the following section on the general public provide a plain verbal description of the worksheets and discuss chemical specific data that are used in the worksheets.

Two types of exposure assessments are considered: general and accidental/incidental. The term *general* exposure assessment is used to designate those exposures that involve estimates of absorbed dose based on the handling of a specified amount of a chemical during specific types of applications. The accidental/incidental exposure scenarios involve specific types of events that could occur during any type of application. The exposure assessments developed in this section as well as other similar assessments for the general public (Section 3.2.3) are based on the typical application rate of 0.4 lbs a.i./acre (Section 2).

3.2.2.1. General Exposures – As described in SERA (2001), worker exposure rates are expressed in units of mg of absorbed dose per kilogram of body weight per pound of chemical handled. These estimates are derived from biomonitoring studies – i.e., studies in which the estimates of absorbed dose are based on measurements of the amount of pesticides excreted by workers. Based on analyses of several different pesticides using a variety of application methods, default exposure rates are estimated for three different types of applications: directed foliar (backpack), boom spray (hydraulic ground spray), and aerial. The general exposure rates that are typically used for each group of workers are:

directed foliar	0.003	(0.0003 - 0.01)	mg/kg per lb a.i. handled/day
boom spray	0.0002	(0.00001 - 0.0009)	mg/kg per lb a.i. handled/day
aerial	0.00003	(0.000001 - 0.0001)	mg/kg per lb a.i. handled/day.

where the first value is the mean or typical estimate of the exposure rate and the values in parentheses are the 95% confidence intervals.

Very little information is available on worker exposure to imidacloprid; the only study directly involving imidacloprid is that of Calumpang and Medina (1996). In this study, workers in the Philippines applied imidacloprid (Confidor 100 SL) as a liquid spray to mangoes for the control of leafhoppers. The application is specified only as "... 2.0 tbsp/100 L for approximately 3 hours per day". Total dermal exposures for these workers was estimated based on deposition to range

from 0.0015 mg/worker per day to 0.0076 mg/kg worker per day. Because the amount of imidacloprid handled by each worker is not specified, these values cannot be compared to the general exposure rates used in most Forest Service risk assessments.

In a submission to the U.S. EPA, Eberhart (1992) provides dermal exposure estimates for workers applying imidacloprid at 0.0302 mg/kg per lb a.i. handled for liquid spray applications to turf, 0.000003 to 0.00054 mg/kg per lb a.i. handled for liquid spray of row crops, 0.000025 to 0.00689 mg/kg per lb a.i. handled for liquid spray of fruit crops, and 0.00002 to 0.0001 mg/kg per lb a.i. handled for granular applications to row crops. All of these estimates, however, are based on the use of surrogate chemicals and the submission provides no data on applications of imidacloprid itself. The lack of more specific and directly useful information on potential worker exposures to imidacloprid does have a substantial impact on the current risk assessment because of the number of different application methods that may be used.

As discussed in Section 2, the application methods used in Forest Service programs most commonly involve tree injection and soil injection. No quantitative information is available on worker exposures associated with tree injection. Except in cases of accidental exposure (Section 3.2.2.2), tree injection would appear to present a very low risk to workers. This may be why the application of pesticides by tree injection is not covered by the U.S. EPA's Worker Protection Standard for Agricultural Pesticides (U.S. EPA 2005). In the absence of specific data on exposure rates associated with tree injection, no general exposure assessment is conducted for this application method. As detailed further in the risk characterization (Section 3.4), there is little basis for asserting that workers involved in broadcast application are at substantial risk, and the risk to workers involved in tree injection applications is probably lower.

Unlike tree injection, soil injection is specifically covered by EPA's Worker Protection Standard for Agricultural Pesticides (U.S. EPA 2005), and it seems more likely that soil injection applications could be associated with exposure rates that are higher than those for tree injection. The only study on tree injection encountered in the literature is that of Fenske and Elkner (1990), who estimated worker-absorbed doses of 0.0095 mg/kg/day in sub-slab and soil injection applications of chlorpyrifos around houses. Because of the nature of these applications (as with many soil injection applications), application rates in terms of lbs a.i. per gallon are not specified and thus this value is not directly comparable to exposure rates used in most Forest Service risk assessments. In the workbook for soil injection that accompanies the current risk assessment, all of the dose rates typically used for worker exposure assessments – i.e., directed foliar, broadcast ground and aerial applications – are included in Worksheets C03a-c, with the standard assumptions on the area treated. While this may be coincidental, the central estimate of the absorbed dose for backpack workers (Worksheet C0a) is 0.00525 mg/kg/day, reasonably close to the estimate of 0.0095 mg/kg/day from the study by Fenske and Elkner (1990). In the absence of any better data, the dose estimates for backpack workers are used in the current risk assessment to characterize potential risks to workers involved in soil injections of imidacloprid.

For broadcast applications, the standard exposure rates given in SERA (2001) and specified at the start of this section are used for both liquid and granular applications. The use of these values for liquid formulations is standard in most Forest Service risk assessments. As specified

in SERA (2001), these rates are based on a large number of worker studies, most of which involve applications of liquid formulations. There is less certainty in the use of these exposure rates for granular formulations. Nonetheless, in risk assessments of other agents covered by Forest Service risk assessments – e.g., 2,4-D and hexazinone – exposure rates for liquid and granular applications appear to be comparable. In addition, Pesticide Handlers Exposure Database (an exposure assessment model used by U.S. EPA) indicates that deposition of pesticides onto the skin will be comparable for both liquid formulations and granular formulations.

3.2.2.2. Accidental Exposures – Typical occupational exposures may involve multiple routes of exposure (i.e., oral, dermal, and inhalation); nonetheless, dermal exposure is generally the predominant route for pesticide applicators (Ecobichon 1998; van Hemmen 1992). Typical multi-route exposures are encompassed by the methods used in Section 3.2.2.1 on general exposures. Accidental exposures are most likely to involve splashing a solution of pesticide into the eyes or various dermal exposure scenarios.

As summarized in Section 3.1.11.3, imidacloprid does not appear to be an eye irritant, although some formulations of imidacloprid may cause mild to moderate eye irritation. Quantitative methods for characterizing hazard based on accidental exposures associated with splashing a solution of a chemical into the eyes or of dust from granular formulations getting into the eyes have not been developed. Consequently, accidental exposure scenarios of this type are considered only qualitatively in the risk characterization (Section 3.4).

There are various methods for estimating absorbed doses associated with accidental dermal exposure (U.S. EPA/ORD 1992; SERA 2001). Two general types of exposure are modeled: those involving contact with a solution of the pesticide on contaminated clothing and those associated with accidental spills of the pesticide onto the surface of the skin. Any number of specific exposure scenarios could be developed for direct contact or accidental spills by varying the amount or concentration of the chemical on or in contact with the surface of the skin and by varying the surface area of the skin that is contaminated.

For the liquid formulation covered in this risk assessment (e.g., Provado 1.6), two exposure scenarios are developed for each of the two types of dermal exposure, and the estimated absorbed dose for each scenario is expressed in units of mg chemical/kg body weight. Both sets of exposure scenarios are summarized in Worksheet E01 of the workbook for liquid formulations, with references to other worksheets in which the specific calculations are detailed. For the granular formulations, *spills* on to the hands or legs are not a meaningful scenario. Hands, legs, or other parts of the body may become contaminated with imidacloprid in the normal course of use, and this is discussed in the previous subsection. For accidental exposures, dust from granular formulations may be deposited on the skin. These exposures are estimated based on zero-order absorption, as discussed further in this section.

Exposure scenarios involving direct contact with solutions of the chemical are characterized by immersion of the hands for 1 minute or wearing contaminated gloves for 1 hour. Generally, it is not reasonable to assume or postulate that the hands or any other part of a worker will be

immersed in a solution of a pesticide for any period of time. On the other hand, contamination of gloves or other clothing is quite plausible. For these exposure scenarios, the key element is the assumption that wearing gloves grossly contaminated with a chemical solution is equivalent to immersing the hands in a solution. In either case, the concentration of the chemical in solution that is in contact with the surface of the skin and the resulting dermal absorption rate are essentially constant.

Exposure scenarios involving chemical spills onto the skin are characterized by a spill on to the lower legs as well as a spill on to the hands. In these scenarios, it is assumed that a solution of the chemical is spilled on to a given surface area of skin and that a certain amount of the chemical adheres to the skin. The absorbed dose is then calculated as the product of the amount of the chemical on the surface of the skin (i.e., the amount of liquid per unit surface area multiplied by the surface area of the skin over which the spill occurs and the concentration of the chemical in the liquid), the first-order absorption rate, and the duration of exposure. This may be one of the few exposure scenarios that could be applicable to tree-injection applications – i.e., the accidental rupture of a capsule containing a solution of imidacloprid that might contaminate the skin. While a specific workbook is not provided for tree injections of imidacloprid, this scenario is encompassed by the corresponding scenarios used for liquid or granular broadcast applications of imidacloprid.

The methods used in developing these accidental dermal dose estimates are typically applied only to liquid formulations. For granular formulations, no standard methods for estimating exposure are available. Nonetheless, granular imidacloprid on the surface of the skin might be regarded as analogous to exposure to a neat (undiluted) solution. For such exposures, the U.S. EPA/ORD (1992) recommends using the solubility of the compound in water as an approximation of the concentration of the chemical on the surface of the skin. The rationale for this approach is that the amount of the chemical on the surface of the skin will saturate the pore water of the skin and the limiting factor on the concentration in pore water will be solubility of the chemical in water. As indicated in Table 2-1, the water solubility of imidacloprid is 610 mg/L (Tomlin 2005), which is equivalent to 0.61 mg/mL. As noted in the Worksheets for zero-order absorption for granular formulations (C02a and C02b), the concentrations of imidacloprid used in these exposure assessments is set at the water solubility of imidacloprid.

3.2.3. General Public

3.2.3.1. General Considerations – Under normal conditions, members of the general public should not be exposed to substantial levels of imidacloprid. For tree injection applications, no exposure scenarios are plausible and none are derived in the current risk assessment. For soil injection, exposure to imidacloprid from contaminated water is plausible and these exposures are detailed in the workbook for soil injection in clay and loam soils (Attachment 3) and in sandy soils (Attachment 4).

While the Forest Service does not anticipate using broadcast applications, this risk assessment includes a consideration of exposures associated with broadcast application to clay and loam soils of both liquid formulations (Attachment 1) and granular formulations (Attachment 2). For broadcast applications, any number of exposure scenarios can be constructed for the general

public, depending on various assumptions regarding application rates, dispersion, canopy interception, and human activity. Several standard and highly conservative scenarios are developed for this risk assessment and are detailed in the attachments.

Applications in predominantly sandy soils lead to estimates of exposures via contaminated water that are similar for soil injection as well as broadcast applications of liquid and granular formulations. Consequently, these exposures are detailed in a separate workbook (Attachment 4).

Both acute and chronic exposure scenarios are developed. Most of the acute exposure scenarios involve accidental exposures, and assume that an individual is exposed to the compound either during or shortly after its application. Specific scenarios are developed for direct spray, dermal contact with contaminated vegetation, and the consumption of contaminated water, fish, fruit, and vegetation. Most of these scenarios should be regarded as extreme, some to the point of limited plausibility. The chronic exposure scenarios parallel the acute exposure scenarios for the consumption of contaminated water, fish, fruit, and vegetation, but are based on estimated levels of exposure for longer periods after application.

For some exposure scenarios, distinctions are made between the liquid and granular formulations. As discussed in Section 3.2.2.2 for dermal exposures, accidental spills onto the surface of the skin are not relevant to granular formulations. Thus, the accidental spill Worksheets, D01a and D01b, are included in the worksheets for liquid formulations but omitted in the worksheets for granular formulations.

The most significant quantitative distinction between the granular and liquid formulations involves exposure scenarios involving contaminated vegetation. As discussed further below, residues of imidacloprid on vegetation will be substantially greater with liquid formulations (which may be applied directly to vegetation) than with granular formulations (which will be applied directly to soil). Relative to liquid formulations, these differences lead to much lower estimates of exposure for granular applications in terms of contaminated vegetation, but some higher estimates of exposures for granular applications in terms of contaminated water.

All of the exposure scenarios developed for the general public are summarized in Worksheet E02 of the workbooks. As with the worker exposure scenarios, details of the assumptions and calculations involved in these exposure assessments are given in the worksheets that accompany this risk assessment (Worksheets D01a–D10b). The remainder of this section focuses on a qualitative description of the rationale for and quality of the data supporting each of the assessments.

3.2.3.2. Direct Spray – Direct sprays involving ground applications are modeled in a manner similar to accidental spills for workers (Section 3.2.2.2). In other words, it is assumed that the individual is sprayed with a solution containing the compound and that an amount of the compound remains on the skin and is absorbed by first-order kinetics. For these exposure scenarios, it is assumed that during a ground application, a naked child is sprayed directly with a solution of the pesticide. These scenarios also assume that the child is completely covered (that

is, 100% of the surface area of the body is exposed) (Worksheet D01a). These are extremely conservative exposure scenarios and are likely to represent upper limits of plausible exposure. An additional set of scenarios are included involving a young woman who is accidentally sprayed over the feet and legs (Worksheet D01b). For each of these scenarios, specific assumptions are made regarding the surface area of the skin and body weight, as detailed in Worksheets D01a and D01b (along with the sources used for making the assumptions). These exposures all involve a liquid spray (Attachment 1) and thus are not included in the workbooks for granular formulations (Attachment 2) or soil injection (Attachment 3).

3.2.3.3. Dermal Contact with Contaminated Vegetation – In this exposure scenario, it is assumed that the pesticide is applied at a given rate and that an individual comes in contact with sprayed vegetation or other contaminated surfaces at some period after the spray operation. For these exposure scenarios, some estimates of dislodgeable residue and the rate of transfer from the contaminated vegetation to the surface of the skin must be available. No data on dermal transfer rates are available for imidacloprid so the estimation methods of Durkin et al. (1995) are used as defined in Worksheet D02 of the workbooks for liquid and granular formulations.

Standart (1999) has estimated the dislodgeable foliar residue of imidacloprid at 0.00018 mg/cm^2 to 0.0009 mg/cm^2 after a cumulative application of 0.3 lb a.i./acre. These estimates were based on data from other pesticides applied to cotton, apples, and grapes. Since 0.3 lb a.i./acre corresponds to an application rate of 0.003363 mg/cm^2, the dislodgeable residue as a proportion of the application rate was estimated by Standart (1999) as 0.054 [0.00018 mg/cm^2 / 0.003363 mg/cm^2] to 0.27 [0.0009 mg/cm^2 / 0.003363 mg/cm^2]. These values bracket the standard value of 0.1 used in most Forest Service risk assessments. For the current risk assessment, the standard value of 0.1 is used to estimate dislodgeable residue on turf (Worksheet D02). As discussed in Section 3.4, the hazard quotients associated with this exposure scenario are far below a level of concern, and this assumption has no impact on the current risk assessment.

The exposure scenario assumes a contact period of one hour and assumes that the chemical is not effectively removed by washing until 24 hours after exposure. Other assumptions used in this exposure scenario involve estimates of body weight, skin surface area, and first-order dermal absorption rates, as discussed in the previous section and detailed in Worksheet D03.

This exposure scenario is included in both the worksheets for applications of liquid formulations (Attachment 1) and granular formulations. As discussed further in Section 3.2.3.6, the worksheet for granular applications assumes that the imidacloprid is applied primarily to the soil and the residue on vegetation after granular application is assumed to be 0.01 of the plant residues after directed foliar applications. Again, this has no impact on the risk characterization because the risks associated with directed foliar application are far below a level of concern.

3.2.3.4. Consumption of Contaminated Water – Water can be contaminated from runoff, as a result of leaching from contaminated soil, from a direct spill, or from unintentional contamination from drift during an application. For this risk assessment, three exposure scenarios are considered for the acute consumption of contaminated water: an accidental spill into a small pond (0.25 acres in surface area and 1 meter deep); accidental direct spray of or

incidental drift into a pond and stream; and the contamination of a small stream and pond by runoff, sediment loss, or percolation. In addition, chronic estimates of concentrations in water are based on a combination of modeling and monitoring data. Each of these scenarios are considered in the following subsections.

3.2.3.4.1. Accidental Spill – The accidental spill scenario assumes that a young child consumes contaminated water shortly after an accidental spill into a small pond; specifics are given in Worksheet D05 of the workbooks. Because this scenario is based on the assumption that exposure occurs shortly after the spill, no dissipation or degradation of the pesticide is considered. The actual concentrations in the water would depend heavily on the amount of compound spilled, the size of the water body into which it is spilled, the time at which water consumption occurs relative to the time of the spill, and the amount of contaminated water that is consumed. This scenario is dominated by arbitrary variability and the specific assumptions used will generally overestimate exposure.

For liquid formulations, Forest Service risk assessments use a standard scenario – the spill of 200 gallons of a *field solution* – i.e., the pesticide diluted with water to the concentration that is anticipated in Forest Service programs (Section 2). Based on the spill scenario for a liquid formulation at an application rate of 0.4 lbs/acre, the concentration of imidacloprid in a small pond is estimated to range from about 1.8 mg/L to 7.3 mg/L, with a central estimate of about 3.6 mg/L (Worksheet D05).

For applications of granular formulations and soil injection, no standard exposure scenarios have been developed for the accidental contamination of a small pond. As with liquid formulations, any number of scenarios could be modeled. For the current risk assessment, the worksheets for applications of granular formulations (Attachment 2) and soil injection (Attachment 3) assume that the amount of imidacloprid spilled into a small pond ranges from the amount required to treat one acre (0.4 lbs) to the amount required to treat 100 acres (40 lbs), with a central estimate based on the amount required to treat 10 acres (4 lbs). These are somewhat more extreme scenarios than that used for liquid formulations, and the resulting concentrations in a small pond range from 0.18 mg/L to 18 mg/L with a central estimate of 1.8 mg/L.

3.2.3.4.2. Accidental Direct Spray/drift for a Pond or Stream – These scenarios are less severe but more plausible than the accidental spill scenario described above. The U.S. EPA typically uses a two meter deep pond to develop exposure assessments (SERA 2004). If such a pond is directly sprayed with imidacloprid at the nominal application rate of 0.4 lbs/acre, the peak concentration in the pond would be about 0.022 mg/L, equivalent to 22 µg/L or 22 ppb (Worksheet D10a). This concentration is a factor of about 330 below the upper bound of the peak concentration of 7.3 mg/L after the accidental spill of a liquid formulation, and a factor of about 820 below the upper bound of the peak concentration of 18 mg/L after the accidental spill of a granular formulation. The D10a worksheets also model concentrations at distances of 100 to 500 feet downwind based on standard values adapted from AgDrift (SERA 2005).

Similar calculations can be made for the direct spray of or drift into a stream. For this scenario, the resulting water concentrations will be dependent on the surface area of the stream that is

sprayed and the rate of water flow in the stream. The stream modeled using GLEAMS (see below) is about 6 feet wide (1.82 meters), and it is assumed that the pesticide is applied along a 1038 foot (316.38 meters) length of the stream with a flow rate of 710,000 L/day. Using these values, the concentration in stream water after a direct spray is estimated at about 0.037 mg/L. Much lower concentrations, about 0.00003 mg/L to 0.005 mg/L, are estimated based on drift at distances of 25 to 900 feet (Worksheet 10b).

It should be noted that no distinction is made between the application of liquid and granular formulations. Drift estimates used in Forest Service risk assessments are based on AgDrift, a model developed as a joint effort by the EPA Office of Research and Development and the Spray Drift Task Force, a coalition of pesticide registrants (Teske et al. 2001). AgDrift does not explicitly incorporate options for the application of granular products, and no field data have been encountered on drift of imidacloprid after the application of granular formulations. The extent to which the general drift estimates used for liquid formulations are appropriate for granular applications is unclear. This uncertainty has little direct impact on this exposure scenario, however, because only the direct spray scenario is used quantitatively.

3.2.3.4.3. Gleams Modeling – This section describes the relatively standardized modeling approach used in Forest Service risk assessments. This is followed by subsections on both other modeling efforts and the available monitoring data. Modeling of concentrations in surface water conducted for this risk assessment are based on GLEAMS (Groundwater Loading Effects of Agricultural Management Systems) modeling. GLEAMS is a root zone model that can be used to examine the fate of chemicals in various types of soils under different meteorological and hydrogeological conditions (Knisel and Davis 2000). As with many environmental fate and transport models, the input and output files for GLEAMS can be complex. The general application of the GLEAMS model and the use of the output from this model to estimate concentrations in ambient water are detailed in SERA (2004). The chemical-specific values used in the GLEAMS modeling are summarized in Table 3-2.

In Forest Service programs, imidacloprid will not be applied over a large proportion of a watershed. Imidacloprid applications will generally be restricted to relatively small stands of hemlock. For example, on a 10 acre plot, it is anticipated that no more than 20% of the plot – i.e., 2 acres – would be treated. In riparian areas, stream banks could be treated over a 200 foot distance from the stream (Mistretta 2005).

Another important factor in assessing the potential for contamination of ambient water involves the application method. For tree injection, no substantial contamination of surface water appears likely. The injected imidacloprid will be transported throughout the tree. Needle fall will occur slowly, and concentrations of imidacloprid in fallen needles are likely to be low and not available for transport to surface water. In any event, GLEAMS and other similar environmental fate models do not have the ability to model tree injection and the potential for contamination of surface water can be handled only qualitatively.

GLEAMS is capable of modeling soil injection applications; for this risk assessment, GLEAMS runs were conducted assuming a treatment area of two acres and an injection depth of six inches.

GLEAMS also can accommodate broadcast applications of liquid formulations, and this application method was also modeled for a two acre plot. GLEAMS is not designed specifically to assess the application of granular formulations. Nonetheless, some attempt was made to qualitatively assess plausible differences between the application of liquid formulations and granular formulations. As discussed further in Section 3.2.3.6, one of the major differences between granular formulations and liquid formulations will be the amount that is retained on treated vegetation. For liquid applications, the fraction of the total amount of imidacloprid that is deposited on foliage is taken as 0.5. For granular applications applied directly to soil, a much lower value, 0.01, is used. In an attempt to mimic the slower release of imidacloprid from granular formulations (e.g. Fernandex-Perex et al. 1998), the proportion of clay, organic matter, and silt in top layer of soil for model runs in loam and sand was set to the values typically used for model runs on clay soils (SERA 2004). Other characteristics such as soil porosity or saturated conductivity were not changed, because the number of granules applied in normal applications are not likely to alter these characteristics in normal applications.

The GLEAMS modeling yielded estimates of runoff, sediment, and percolation that were used to calculate concentrations in the stream adjacent to a treated plot, as detailed in Section 6.4 of SERA (2004). As detailed in SERA (2004), rainfall rates are a dominant factor in pesticide transport and GLEAMS runs were made at ten different rainfall rates ranging from 5 to 250 inches per year. Soil texture is another very important factor in the pattern of offsite movement and separate runs were made for clay, loam, and sand. The results of the GLEAMS modeling are summarized in Table 3-3 for a small stream and Table 3-4 for a small pond. Additional details of the modeling output are given in Appendix 10 (broadcast applications of a liquid formulation), Appendix 11 (broadcast applications of a granular formulation), and Appendix 12 (soil injection). Each appendix contains six tables giving the modeled concentrations in a small stream (Table 1), a small pond (Table 2), the top 60 inches of soil column (Table 3), and the top 12 inches of the soil column (Table 4). Additional tables specify the maximum depth of soil penetration (Table 5) and the proportion of the pesticide transported offsite by runoff and sediment losses combined (Table 6). All values are based on a normalized application rate of 1 lb/acre.

No surface water contamination is expected based on the estimates made for very arid regions – i.e., annual rainfall of 10 inches or less. As summarized in Table 3-3 for the pond and Table 3-4 for the stream, the concentrations in surface water are comparable for broadcast applications of liquid formulations and granular formulations. The peak and average concentrations for granular applications tend to be somewhat higher than those for liquid formulations. This is a relatively consistent pattern in modeling comparable applications of liquid and granular formulations and is due primarily to the greater amounts of the pesticide on the soil surface (and thus subject to runoff) after the application of a granular formulation relative to a liquid formulation. This pattern was noted for imidacloprid in a study by Armbrust and Peeler (2002). In general, concentrations of imidacloprid resulting from its application to clay tend to be higher than equal applications made to loam. This is common and is associated with increased runoff and sediment loss from clay compared to loam (see Table 6 in Appendices 10 and 11).

Soil injections of granular or liquid formulations to clay or loam lead to a substantially different estimates of concentration in water compared to broadcast applications. Because soil injection involves placing the chemical substantially below the soil surface (6 inches in the modeling for the current assessment), runoff and sediment losses are essentially zero (see Table 6 in Appendix 12 and compare to the corresponding Table 6 in Appendices 10 and 11). Thus, modeled concentrations of imidacloprid in pond or stream water after soil injection in both clay and loam are negligible. These modeled results are consistent with limited field simulation studies that suggest a very low leaching potential for imidacloprid in loam or sandy loam soils (Bachlechner 1992; Fritz and Brauner 1988; Hellpointner 1994a,b).

When applied to sandy soils, however, soil injection leads to concentrations in water that are comparable to those modeled for broadcast applications of liquid or granular formulations. As summarized in Appendix 8, substantial leaching of imidacloprid has been reported in some studies (Felsot et al. 1984; Flores-Cespedes et al. 2002; Gupta et al. 2002). It seems apparent that imidacloprid can leach significantly under some conditions and that the extent of leaching may be dependent on many factors including the dissolved organic carbon in the soil (Flores-Cespedes et al. 2002), the concentration of imidacloprid in the soil (Oliveira et al. 2000), and the aging of imidacloprid in soils (Oi 1999).

3.2.3.4.4. Other Modeling Efforts – No other attempts to model the concentrations of imidacloprid in water have been encountered.

3.2.3.4.5. Monitoring Data – The only monitoring study identified in the literature is an ongoing ground-water monitoring study, for which preliminary reports have been submitted to the U.S. EPA (Dyer and Helfrich 1999, 2000). In this study, imidacloprid (as Admire 2F) was applied at a rate of 0.34 lb a.i./acre in May of 1996. The total water input (rainfall plus irrigation) was 170 inches through December 1999. Thus, in terms of annual rainfall, the water input corresponded to about 50 inches per year [170 inches ÷ 3.5 years = 48.5 inches per year]. The maximum concentration of imidacloprid detected in ground water was 0.2 ppb and the average concentration was on the order of 0.04 ppb. The reports by Dyer and Helfrich (1999, 2000) do not specify the soil type. As indicated in Appendix 10, these concentrations are in the range of concentrations modeled for clay and loam soils at an annual rainfall rate of 50 inches. These ranges are very wide, so this correspondence may be incidental.

3.2.3.4.6. Concentrations in Water Used for Risk Assessment – A summary of the concentrations of imidacloprid in water that are used for the current risk assessment is given in Table 3-5. This table gives the water contamination rates, the normalized concentrations in water converted to units of ppm or mg/L per lb a.i./acre. These values are used in the worksheets in the various exposure scenarios involving contaminated water in both the human health and ecological risk assessments. In the worksheets, these water contamination rates are multiplied by the application rate to yield estimates of concentrations of imidacloprid in water.

These water contamination rates are based exclusively on the GLEAMS modeling discussed in Section 3.2.3.4.3. As discussed in Section 3.2.3.4.5, there is very little monitoring information

available and the one study that is available cannot be used directly to assess confidence in the concentrations estimated from GLEAMS.

Four sets of values are derived: concentrations after applications of liquid formulations to clay or loam, concentrations after applications of granular formulations to clay or loam, concentrations after soil injection in clay or loam, and concentrations after any application to predominantly sandy soil. As noted in Section 3.2.3.4.3, imidacloprid does not appear to be highly mobile in most soils but there is reasonable concern that it may be highly mobile in sandy soil. For each set of values, the upper range for both peak and longer-term concentrations is taken as the highest value from either the stream or pond modeling, rounded to one significant place. The central estimate is based primarily on values for rainfall rates of 50 inches per year. The lower estimates are somewhat arbitrary but these have no impact on the characterization of risk in either the human health risk assessment (Section 3.4) or the ecological risk assessment. Note that both central and lower estimates of peak and longer-term concentrations after soil injection into clay or loam are set to zero. This approach is taken because the highest modeled concentration for these soil textures after soil injection applications is 4.34×10^{-5} mg/L per lb/acre applied (Appendix 12, Table 2). This water contamination rate is essentially negligible.

3.2.3.5. Consumption of Contaminated Fish – Many chemicals may be concentrated or partitioned from water into the tissues of animals or plants in the water. This process is referred to as bioconcentration. Bioconcentration is generally measured as the ratio of the concentration in the organism to the concentration in the water, and expressed in units of kg/L.

Relatively little information is available on the bioconcentration of imidacloprid. Ding et al. (2004) reports bioconcentration factors of 0.97 to 1.5 L/kg in *Brachydanio rerio* (zebra fish). This study is published in the Chinese literature and the information on the bioconcentration factor is taken from an abstract. Meylan and Howard (2000) report an experimental bioconcentration factor of 3.7 L/kg but the primary source of this information is unclear.

This paucity of information is unusual for a pesticide and may reflect the fact that an RED (Re-registration Eligibility Decision) for imidacloprid is not yet available from the U.S. EPA. Typically, the U.S. EPA will require at least one detailed experimental study on bioconcentration, typically using bluegill sunfish. For the current risk assessment, the higher value of 3.7 L/kg is used and this value is applied to both the human health and ecological risk assessments.

For the acute and chronic exposure scenarios involving the consumption of contaminated fish, the water concentrations of imidacloprid used are identical to the concentrations used in the contaminated water scenarios (Section 3.2.3.4.6). The acute exposure scenario is based on the assumption that an adult angler consumes fish taken from contaminated water shortly after an accidental spill into a pond.

Because of the available and well-documented information and substantial differences in the amount of caught fish consumed by the general public and native American subsistence populations, separate exposure estimates are made for these two groups, as illustrated in

Worksheet D08a and D08b. The chronic exposure scenario is constructed in a similar way, as detailed in Worksheets D09a and D09b.

3.2.3.6. Consumption of Contaminated Vegetation – Although none of the Forest Service applications of imidacloprid will involve the treatment of crops, Forest Service risk assessments typically include standard exposure scenarios for the acute and chronic consumption of contaminated vegetation. Two sets of exposure scenarios are provided: one for the consumption of contaminated fruit and the other for the consumption of contaminated vegetation. These scenarios are detailed in Worksheets D03a and D03b for acute exposure and Worksheets D04a and D04b for chronic exposure. These exposure assessments are used only in the worksheets for broadcast applications of liquid formulations (Attachment 1) and granular formulations (Attachment 2).

In most Forest Service risk assessments, the concentration of the pesticide on contaminated fruit and vegetation is estimated using the empirical relationships between application rate and concentration on different types of vegetation (Fletcher et al. 1994). As detailed in Appendix 9, the available data on vegetation residues of imidacloprid (e.g., Lin 1992a,c,d; Toll 1994) are consistent with the general estimates from Fletcher et al. (1994) and these general values are used in the worksheets.

For all granular formulations applied directly to soil, the residue rates from Fletcher et al. (1994) are multiplied by a factor of 0.01. This is the ratio used in the GLEAMS modeling for granular formulations and is intended as a crude approximation of plausible residues that might incidently contaminate foliar surfaces after soil applications.

For chronic exposures, both initial concentrations and a halftime on vegetation is required to estimate the time-weighted average exposure (Worksheet D04). In these worksheets, a foliar halftime of 10 days is used based on the reported halftime of 9.8 days on turf from Lin (1992a,c). Much shorter halftimes (about 1 day) have been reported (Lin 1992d) on foliage from potatoes. The use of the 10 day halftime is more conservative (i.e., leads to higher estimates of concentrations on vegetation). This conservative approach does have a minor impact on the risk characterization for liquid formulations and this is discussed further in the risk characterization (Section 3.4).

Much of the efficacy of imidacloprid for the control of adelgids depends upon its uptake into plants and its subsequent translocation to where it can be consumed by the adelgids. This also results in exposures from the consumption of vegetative parts from plants growing in treated soil. Translocation of imidacloprid into plant tissues potentially subject to human consumption may also elevate the exposure estimates after broadcast applications of granular formulations of imidacloprid. While studies are available on the translocation of imidacloprid after foliar application (Buchholz and Nauen 2002; Weichel and Nauen 2004) as well as seed treatment (Westwood et al. 1998), there is not sufficient information to estimate concentrations of imidacloprid in edible plants after soil applications or tree injection. In pine, oak, and hemlock treated with imidacloprid by tree or soil injection at rates appropriate for the control of adelgids, Tattar et al. (1998) noted peak concentrations in foliage of about 1 to 2.5 mg/kg over 12 to 20

week intervals after treatment. As noted in the worksheets for liquid formulations (Attachment 1), concentrations in vegetation after direct spray are in the range of about 3 to 6 mg/kg for fruit and about 18 to 54 mg/kg for broadleaf vegetation. Thus, is appears that the hazard quotients for the consumption of contaminated vegetation as the result of soil or tree injection will be encompassed by the exposure scenarios for the direct spray of fruit using liquid formulations of imidacloprid.

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview

Acute and chronic risk values are derived for imidacloprid. Following standard practices for USDA risk assessments, risk assessment values available from U.S. EPA are adopted. U.S. EPA has derived a chronic RfD for imidacloprid of 0.057 mg/kg/day. This chronic RfD is well-documented and is used directly for all longer term exposures to imidacloprid. This value is based on a NOAEL of 5.7 mg/kg/day in rats and an uncertainty factor of 100 – two factors of 10 for interspecies and intraspecies variability.

U.S. EPA has derived an acute RfD for imidacloprid of 0.14 mg/kg/day. This value is based on a LOAEL of 42 mg/kg in rats and an uncertainty factor of 300 - a factor of three for extrapolating NOAEL from LOAEL, and two factors of 10 for interspecies and intraspecies variability. Acute studies have shown that the WAK 3839 metabolite of imidacloprid is much less toxic than imidacloprid (Appendix 1).

3.3.2. Chronic RfD

The most recent RfD for imidacloprid is 0.057 mg/kg/day, a value derived by the U.S. EPA's Office of Pesticide Programs (U.S. EPA/OPP 2003). This compound is not listed on the U.S. EPA's agency-wide list of approved RfDs (i.e., IRIS). As noted in section 3.1.2 and detailed in Appendix 2, most studies conducted with mice and rats report retardation of growth expressed as body weight gain. These changes tend to be transient at the lower doses and shorter periods of imidacloprid administration (Appendix 1) but are more pronounced and consistent at higher doses or following longer periods of administration (Eiben 1988a,b,1989, 1991; Watta-Gebert 1991a, b; Eiben and Kaliner 1991). It is not possible to attribute these changes solely to reduced food consumption (e.g., the decrease in body weight is accompanied by reduced food consumption in some studies but significantly increased food consumption in other studies). The mechanism of the decrease in body weight cannot be clearly determined. It could be related to changes in the liver (increases or decreases in plasma cholesterol; altered glucose concentrations; enzyme induction and multi-focal group cell necrosis [higher doses]), changes in metabolic rate (e.g., effects on the thyroid), or to degenerative changes in the tissues and organs related to the digestive system (e.g., degeneration of salivary glands in the study by Bloch 1987). The most sensitive endpoint observed in any of the available studies is mineralization of the thyroid colloid in male Wistar rats (Eiben and Kaliner 1991; Eiben 1991). Adverse effects on the thyroid were also observed in a study with dogs (Bloch 1987) but only at much higher dietary concentrations than in the rat studies.

The RfD derived by the U.S. EPA/OPP (2003) is based on studies by Eiben and Kaliner (1991) and Eiben (1991). In the first study, male and female Wistar rats were fed dietary concentrations of 0, 100, 300 and 900 ppm technical grade imidacloprid for 24 months (Appendix 2). These dietary concentrations correspond to mean measured doses of 0, 5.7, 16.9 and 51.3 mg/kg body weight per day for males and 0, 7.6, 24.9 and 73.0 mg/kg body weight per day for females. Treatment-related increases in the incidence of mineralization of the colloid of the thyroid follicles was observed in males at 300 and 900 ppm, and in females at 900 ppm. Treatment-related reductions in body weight gain were observed at 900 ppm in both sexes. The second study by Eiben (1991) confirmed the effects on body weight and the thyroid (Appendix

2). Groups of male and female Wistar rats were fed 0 or 1800 ppm technical grade imidacloprid in the diet for 24 months. This corresponded to doses of 0 and 102.6 mg/kg/day for males, and 0 and 143.7 mg/kg/day for females. An increased incidence of thyroid changes (mineralization of colloid; fewer colloid aggregation sites; parafollicular hyperplasia sites with minimal intensity) and reduction in body weight gain were observed in both sexes.

U.S. EPA divided the NOAEL of 5.7 mg/kg/day (males, Eiben and Kaliner 1991) by an uncertainty factor of 100 to arrive at the chronic RfD of 0.057 mg/kg/day. The uncertainty factor of 100 accounts for inter- and intra-species variability.

Under the Food Quality Protection Act (FQPA), the U.S. EPA is required to consider an additional uncertainty factor of 10 for the protection of infants and children. For imidacloprid, the U.S. EPA/OPP (2003) determined that the additional uncertainty factor is not required because of the information indicating that imidacloprid does not have developmental or reproductive effects at doses below those associated with the observed thyroid effects. As such, the RfD derived on the basis of thyroid effects will also be protective of developmental and reproductive effects.

3.3.4. Acute RfD

U.S. EPA/OPP (2003) derived an acute RfD of 0.14 mg/kg on the basis of an acute LOAEL of 42 mg/kg for decreased measures of motor and locomotor activity in female rats. The study from which the LOAEL was derived (Sheets 1994 a,b) is an acute oral neurotoxicity screening study, in which male and female Sprague-Dawley rats were exposed to technical grade imidacloprid by gavage at doses of 0, 42, 151 and 307 mg/kg body weight (Appendix 1). A supplemental study was conducted in which rats were given gavage doses of technical-grade imidacloprid at 0 (vehicle control) or 20 mg/kg body weight (Appendix 1). No mortality, clinical signs, neurological effects, or effects on body weight were observed at 20 mg/kg.

U.S. EPA chose to derive the acute RfD on the basis of the LOAEL of 42 mg/kg rather than the NOAEL of 20 mg/kg. Dividing the LOAEL of 42 mg/kg by an uncertainty factor of 300 (3 for NOAEL to LOAEL extrapolation; 10 for interspecies variability; 10 for intraspecies variability), U.S. EPA/OPP (2003) derives an RfD of 0.14 mg/kg.

U.S. EPA/OPP (2003) notes that the NRDC (Natural Resources Defense Council) criticized the use of the LOAEL of 42 mg/kg as the basis for deriving the acute RfD. In response to this criticism, the U.S. EPA/OPP (2003) cites a dietary study in which doses equivalent to 10, 70, and 200 mg/kg/day were not associated with any changes in motor activity. The U.S. EPA/OPP (2003) does not provide a citation for this study. A dietary study in rats that would correspond to the equivalent doses cited by U.S. EPA/OPP (2003) is not apparent in Appendix 1 and Appendix 2. Nonetheless, the acute RfD derived by U.S. EPA appears reasonable. As summarized in Appendix 1, Sheets (1994a) conducted a supplemental study in rats in which no adverse effects were observed after a single gavage dose of 20 mg/kg. The uncertainty factor of 3 to adjust for the LOAEL does results in a lower RfD than if the supplemental NOAEL of 20 mg/kg had been used directly – i.e., the acute RfD would correspond to 0.2 mg/kg rather than 0.14 mg/kg.

3.4. RISK CHARACTERIZATION

3.4.1. Overview

The risk characterization for potential human health effects is influenced by the application method. For soil injection and tree injection (i.e., the application methods that are likely to be used by the Forest Service), the risk characterizations for workers and members of the general public are reasonably unequivocal. None of the acute or longer term hazard quotients exceed 1, the level of concern. For members of the general public, the hazard quotients are below the level of concern by factors of 30 million to 10 billion. Workers are likely to be subject to higher levels of exposure. Nonetheless, the highest hazard quotient for workers involved in soil injection is below the level of concern by a factor of about 14. Explicit risk characterizations for tree injection are not made. This is a very selective application method and levels of exposure for workers and members of the general public are likely to be lower (and probably much lower) than those associated with soil injection.

Although the Forest Service does not anticipate using broadcast applications of imidacloprid, these application methods are considered in this risk assessment because other organizations working in cooperation with the Forest Service may consider using broadcast applications of either granular or liquid formulations. In broadcast applications, some exposure scenarios result in modest excursions about the level concern. For workers, the upper range of exposures during the normal broadcast application of either granular or liquid formulations lead to hazard quotients of 1.1. For members of the general public, the highest hazard quotient for non-accidental exposures is 1.5 and this hazard quotient is associated with the upper bound of plausible exposures for the longer-term consumption of contaminated vegetation. Whether members of the general public might actually consume vegetation contaminated with imidacloprid is unclear. Broadcast applications of imidacloprid will not be applied intentionally to crops or other types of vegetation that humans might consume. The intent of broadcast applications will be to apply the imidacloprid to the target vegetation – i.e., hemlocks. Any contamination of vegetation that humans might consume would be unintentional and probably incidental.

Hazard quotients for accidental exposures associated with spills into a small body of water result in hazard quotients with upper bounds that range from 1.1 (adult male consuming fish) to 15 (a child consuming 1 liter of contaminated water). The amounts spilled are set at the amounts required to treat from one acre (0.4 lbs) to100 acres (40 lbs). These assumptions are completely arbitrary and may be unrealistic. Given the relatively small areas that the Forest Service treats with imidacloprid, it seems highly unlikely that the amount required to treat 100 acres would be assembled in one container or vehicle and would then be spilled into a small pond. This exposure scenario is intended simply to illustrate the different consequences of spilling different amounts of imidacloprid. Any reasonable assessment of risk would need to be based on site-specific information of an actual spill.

3.4.2. Workers

A quantitative summary of the risk characterization for workers is presented in Worksheet E02 of the imidacloprid workbooks. This worksheet is contained in the workbooks for broadcast liquid applications (Attachment 1), broadcast granular applications (Attachment 2), and soil injection (Attachment 3).

For workers as well as members of the general public, the quantitative risk characterization is expressed as the hazard quotient, the ratio of the estimated exposure from Worksheet E01 to the RfD. For acute accidental/incidental exposures, the acute RfD of 0.14 mg/kg derived by U.S. EPA/OPP (2003) is used (Section 3.3.4). For longer term general exposures – i.e., exposures that could occur over the course of several days, weeks, or months during an application season – the chronic RfD of 0.057 mg/kg/day, also derived by U.S. EPA/OPP (2003), is used (Section 3.3.2).

For soil injection and tree injection (i.e., the application methods that are likely to be used by the Forest Service), the risk characterization for workers is reasonably unequivocal. None of the acute or longer term hazard quotients exceed 1, the level of concern. It should be noted, however, that standard worker exposure rates are not available for either soil injection or tree injection. As an alternative, the exposure rate for backpack applications was used for soil injection. Based on the processes involved in soil injection compared to processes involved in backpack applications, it is likely that the actual worker exposures for soil injection are overestimated, and probably grossly overestimated, by using the exposure rate for backpack applications. The highest hazard quotient for workers involved in soil injection is 0.07, below the level of concern (i.e., HQ=1) by a factor of about 14.

As noted in Section 3.2.2.1, no explicit exposure assessment is conducted for tree injection applications. It is likely that tree injection applications will involve negligible exposure to the worker under normal circumstances because the imidacloprid is contained within a capsule or injection device. Nonetheless, accidental exposures such as the rupture of an imidacloprid capsule are conceivable. As noted in the Worksheet E02 for liquid applications (Attachment 1), the upper range of exposures for wearing gloves saturated with imidacloprid for one hour is 0.2, below the level of concern by a factor of 5. This exposure is probably much higher than any plausible exposure for a worker applying imidacloprid by tree injection.

Although the Forest Service does not anticipate using broadcast applications of imidacloprid, some groups that work in cooperation with the Forest Service may consider broadcast application. For such applications, standard worker exposure rates are used, as detailed in Section 3.2.2.1. At an application of 0.4 lb/acre, the highest labeled application rate for any single application, the upper range of the hazard quotient is 1.1 for liquid or granular applications. This is associated with ground broadcast applications. All other hazard quotients are below the level of concern.

3.4.3. General Public

A quantitative summary of the risk characterization for the general public is presented in Worksheet E04 of the imidacloprid workbooks. This worksheet is contained in the workbooks for broadcast liquid applications (Attachment 1), broadcast granular applications (Attachment 2), soil injection applications (Attachment 3), and applications to predominantly sandy soil (Attachment 4). As discussed in Section 3.2.3.4, the application of imidacloprid to predominantly sandy soils using soil injection or broadcast applications of liquid or granular formulations could lead to similar and relatively high concentrations in ambient water compared to applications to predominantly loam or clay soils. Soil type and texture, however, have no impact on exposure scenarios that do not involve contaminated water – e.g., consumption of contaminated vegetation or accidental spray. Thus, Attachment 4 includes only those exposure scenarios for the consumption of contaminated water or fish after applications to sand. Other exposure scenarios are covered in Attachments 1, 2, and 3. As with the risk characterization for workers, risk is expressed quantitatively as the hazard quotient using the RfD values derived by U.S. EPA/OPP (2003) – i.e., the acute RfD of 0.14 mg/kg (Section 3.3.4) or the chronic RfD of 0.057 mg/kg/day (Section 3.3.3).

Also, as with the risk characterization for workers, hazard quotients associated with non-accidental exposures after soil injection or tree injection applications (i.e., the application methods that are most likely to be used by the Forest Service) are negligible (Worksheet E04, Attachment 3). The hazard quotients range from $1x10^{-10}$ (longer term consumption of contaminated fish) to $3x10^{-7}$ (acute consumption of contaminated water) and are below the level of concern by factors of 30 million to 10 billion.

Hazard quotients for accidental exposures associated with spills into a small body of water result in hazard quotients with the upper bounds that range from 1.1 (adult male consuming fish) to 15 (a child consuming 1 liter of contaminated water). The plausibility of these exposure scenarios, however, is unclear. As detailed in Section 3.2.3.4.1, soil injection is not a common method of application and standardized accidental exposure scenarios for this application method have not been used in previous Forest Service risk assessments. For the scenario involving the accidental spill into a body of water, the assumption is that the amount spilled is ranges from the amount required to treat one acre (0.4 lbs) to the amount required to treat 100 acres (40 lbs), with a central estimate based on the amount required to treat 10 acres (4 lbs). These assumptions are completely arbitrary and may be unreasonable. Given the relatively small areas that the Forest Service treats with imidacloprid (Section 2), it seems highly unlikely that the amount required to treat 100 acres would be assembled in one container or vehicle and would then be spilled into a small pond. This should be considered in interpreting the hazard quotients for accidental exposure in assessing any site specific application.

For broadcast applications to predominantly clay or loam soils of liquid formulations (Attachment 1) or granular applications (Attachment 2), hazard quotients are generally below a level of concern for the non-accidental scenarios. The only exception involves the exposure scenario for the longer-term consumption of contaminated vegetation after the broadcast application of a liquid formulation. For this scenario, the upper bound of the hazard quotient is

1.5, modestly above the level of concern (i.e., HQ=1). For the corresponding acute exposure scenario, the upper range of the hazard quotient is 4.

The extent to which a longer-term hazard quotient of 1.5 would present any significant hazard cannot be clearly characterized. As discussed in Section 3.3.2, the experiment on which the chronic RfD is based (Eiben and Kaliner 1991) defined a dietary NOAEL of 100 ppm. The corresponding LOAEL was 300 ppm, a level associated with effects on the thyroid in male rats but not female rats. This LOAEL would correspond to a hazard quotient of 3. The thyroid effect was seen in female rats but only at a dietary concentration of 900 ppm (i.e., an HQ of 9). As noted in Section 3.3.4, the acute RfD is based on a LOAEL (decreased measures of motor and locomotor activity in female rats at a dose of 42 mg/kg bw) using an uncertainty factor of 3 to approximate a NOAEL.

These comparisons of NOAEL and LOAEL values to HQs are not intended to imply a direct correspondence. Because of the uncertainty factors used to derive an RfD as well as the uncertainties in using data on experimental mammals to assess effects in human, an HQ of 3 in males and 9 in females might not be associated with thyroid effects. These comparisons are simply a way, given the available information, of suggesting the potential for adverse effects above the RfD. For a hazard quotient is 1.5, the exposure is intermediate between the NOAEL and LOAEL – i.e., a factor of 2 below that of the corresponding LOAEL.

Another factor that should be considered in interpreting the longer-term HQ of 1.5 involves the exposure assessment. The HQ of 1.5 is based on the consumption of leafy vegetation based on standard residue rates from Fletcher et al. (1994). As noted in Section 3.2.3.6, these residue rates do appear to be reasonable and are consistent with monitored residues of imidacloprid on vegetation. Note that the upper range of the hazard quotient for the corresponding exposure scenario for contaminated fruit is only 0.2. This reflects the lower residue rates that are anticipated on fruit compared to leafy vegetation (Fletcher et al. 1994). Whether members of the general public might actually consume vegetation contaminated with imidacloprid is unclear. Even broadcast applications of imidacloprid will not be intentionally applied to crops or other types of vegetation that humans might consume. The intent of broadcast applications will be to apply the imidacloprid to the target vegetation – i.e., hemlocks. Any contamination of vegetation that humans might consume would probably be incidental.

The simplest verbal interpretation of the hazard quotients is to view them as relative measures of potential risk that can help to identify the types of exposures that might be of greatest concern. For broadcast applications of imidacloprid to clay or loam soils, the consumption of contaminated vegetation is the scenario of greatest concern for non-accidental exposures. For accidental exposure, the greatest concern involves accidental spills into small bodies of water. As noted above in the discussion of soil injection applications, the accidental spill scenarios used for the consumption of contaminated water are standard for broadcast applications but are nonetheless arbitrary.

3.4.4. Sensitive Subgroups

As with exposures to almost any chemical, there is particular concern for children, women who are pregnant or may become pregnant, the elderly, or individuals with any number of different diseases. Nonetheless, there are no reports in the literature suggesting subgroups that may be unusually sensitive to imidacloprid exposure.

As noted in Section 3.1 (Hazard Identification), short-term exposures to high doses of imidacloprid are associated with transient cholinergic effects (dizziness, apathy, locomotor effects, labored breathing) and transient growth retardation. For longer term, lower-dose exposures, effects may occur on the liver, thyroid, and body weight (reduction). The U.S. EPA/OPP (2003) did explicitly incorporate uncertainty factors of 10 for sensitive individuals in the derivations of both the acute and chronic RfDs. Based on the low hazard quotients for workers (Section 3.4.2) and members of the general public (Section 3.4.3), it is not clear that any particular group would be at increased risk from plausible exposures to imidacloprid from Forest Service programs.

3.4.5. Connected Actions

The Council on Environmental Quality (CEQ, which provides the framework for implementing NEPA, defines connected actions (40 CFR 1508.25) as actions which occur in close association with the action of concern; in this case, the use of a pesticide. Actions are considered to be connected if they: (i) Automatically trigger other actions which may require environmental impact statements; (ii) Cannot or will not proceed unless other actions are taken previously or simultaneously, and (iii) Are interdependent parts of a larger action and depend on the larger action for their justification. Within the context of this assessment of imidacloprid, "connected actions" include actions or the use of other chemicals which are necessary and occur ***in close association*** with use of imidacloprid.

As discussed in detail in Sections 3.1.14 (Inerts and Adjuvants) and 3.1.15 (Impurities and Metabolites), imidacloprid formulations contain inert components and impurities which may have an impact on risks to human health and the environment. The available studies discussed throughout this document demonstrate that the presence of the impurities and metabolites in imidacloprid formulations have an insignificant impact on health risk. In the one case of human poisoning discussed in detail in Section 3.1.14, the observed toxicity was attributed to the so-called inert ingredient N-methyl-pyrrolidone.

3.4.6. Cumulative Effects

This assessment considers known chemical interactions or actions, which taken in consideration with the proposed pesticide use, would affect the quality of the human environment (i.e. modify risks to human health and ecological receptors within the context of the risk assessment).

It is beyond the scope of the current risk assessment to identify and consider all agents that might interact with, or cause cumulative effects with imidacloprid. To do so quantitatively would require a complete set of risk assessments on each of the other agents that would be considered. Cumulative effects, within the context of the Food Quality Protection Act (requires assessment

of chemicals with a similar mode of action), have been addressed by the U.S. EPA in their most recent set of food tolerances for imidacloprid:

> *EPA does not have, at this time, available data to determine whether imidacloprid has a common mechanism of toxicity with other substances or how to include this pesticide in a cumulative risk assessment. Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, imidacloprid does not appear to produce a toxic metabolite produce other substances* [Sic]. *For the purposes of this tolerance action, there EPA has not assumed that imidacloprid has a common mechanism of toxicity with other substances.* – U.S. EPA/OPP, 2005a.

As noted in Section 3.1.16, imidacloprid has been shown to induce liver enzymes such as cytochrome P-450. Cytochrome P-450 is a very important enzyme in the metabolism of many endogenous as well as xenobiotic compounds. It is possible that the toxicity of imidacloprid may be affected by and could affect the toxicity of many other agents that either induce or inhibit cytochrome P-450. The nature of the potential effect (i.e., synergistic or antagonistic) would depend on the specific compound and perhaps the sequence of exposure.

The current Forest Service risk assessment specifically considers the effect of repeated exposures to imidacloprid for both workers and members of the general public. The chronic RfD is used as an index of acceptable longer-term exposures. Consequently, the risk characterizations presented in this risk assessment for longer-term exposures specifically address and encompass the potential impact of the cumulative effects of imidacloprid. It should be noted that imidacloprid is applied only once annually by the Forest Service. Given the relatively short half-life of imidacloprid, exposure for workers is in reality likely to be restricted to the day of application. With respect to hypothetical nearby residents, the consumption of contaminated water from an accidental spill or the consumption of contaminated fruits and vegetables is the only major foreseeable scenarios involving repeated exposure.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview

The toxicity of imidacloprid has been well-studied in mammals, birds, terrestrial invertebrates and aquatic organisms, and the mechanism of action is fairly well known. In all species, the toxicity of imidacloprid metabolites is equivalent to or less than that of the parent compound. The nitrosoimine metabolite, a contaminant of imidacloprid preparations (as much as 30%) and a product of imidacloprid metabolism, is of low toxicity to mammals. The predominant metabolites associated with toxicity in insects are olefinic-, dihydroxy- and hydroxy-imidacloprid.

In mammals, the primary chronic toxic effects of imidacloprid are on body weight and the thyroid, although neurotoxic effects have been observed in acute studies following high-dose exposures. In birds, imidacloprid causes neurotoxicity and adverse effects on hatchling growth, and there is evidence that birds learn to avoid imidacloprid-treated seed. Birds appear to be more sensitive to imidacloprid than mammals.

The body of literature on the effects of imidacloprid on insects is large and diverse. There is a general pattern of toxicity following imidacloprid exposure, involving an immediate onset of neurotoxicity, followed by a delayed mortality, usually 4 hours to several days after exposure. Evidence suggests that unchanged imidacloprid may be responsible for the initial neurotoxicity, while the olefinic, hydroxy- and dihydroxy- metabolites which appear at approximately 4 hours post-exposure may be responsible for mortality.

The effects of imidacloprid on beneficial predatory arthropods appears to depend upon the species, and the conditions and rate of application. The parasitic hymenopterans appear to be most sensitive, while ants are most tolerant. In honey bees, imidacloprid at very low doses has been shown to cause mortality and adverse effects on laboratory-conditioned behavioral responses associated with feeding. However, adverse impacts of imidacloprid on foraging and colony vitality under field conditions have yet to be demonstrated. In fact, key studies suggest that imidacloprid may not induce the same learned avoidance behavior in honey bees that have been demonstrated in birds.

Fish, amphibians and aquatic algae are less sensitive to imidacloprid than certain aquatic invertebrates in terms of survival and growth. Among aquatic invertebrates, arthropods such as chironomid and mysid species are extremely sensitive to imidacloprid exposure, with observed adverse effects on survival, growth and reproductive success.

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 mechanism of action of imidacloprid as a nicotinic acetylcholinesterase agonist has been well studied. However, the greatest adverse effects associated with imidacloprid exposure among mammals do not involve effects on acetylcholinesterase or neurotoxicy. The standard acute (Appendix 1) and subchronic or chronic (Appendix 2) toxicity studies (e.g. EPA guideline

studies) conducted on experimental mammals suggest that the greatest effects are on body weight (reduction) and the thyroid at low doses. Neurotoxic effects were observed in acute studies following high-dose exposure. Doses that caused maternal toxicity were also associated with developmental toxicity in rats and rabbits. A developmental neurotoxicity study in rats demonstrated that adverse neurological effects (deficit in performance in the figure-eight maze) could occur among the offspring of imidacloprid-exposed mothers who had no adverse effects following exposure.

On the basis of acute mortality, the available studies suggest that technical grade imidacloprid is more toxic than imidacloprid formulations, and more toxic than its nitrosoimine metabolite (not the des-nitro metabolite) which is sometimes found in food commodities. The lowest LD_{50} value for technical grade imidacloprid, 131 mg/kg body weight, was observed in male mice (Bomann1989b). The lowest LD_{50} value for the nitrosoimine metabolite (NTN 37571 or WAK 3839), 200 mg/kg, was observed in fasted male and female mice (Nakazato 1988a). The LD_{50} values and other endpoints for acute mammalian toxicity in association with imidacloprid formulations used by the Forest Service are summarized in Table 3-1. On the basis of the observed LD_{50} values, imidacloprid and its nitrosoimine metabolite are classified by EPA as slightly to moderately toxic.

In experimental mammals, signs of acute toxicity occurred at doses lower than those causing mortality, regardless of the species, formulation or metabolite administered. Clinical signs of toxicity, including staggering gait, sedation, apathy, tremors, labored breathing and convulsions (higher doses) were apparent shortly after dosing, but were resolved in all animals prior to the end of the study (day 14). Transient decrease in body weight was also a common symptom of imidacloprid-treated animals. From these studies by Sheets, the acute LOAEL of 42 mg/kg (females, reduced locomotor/motor activity) was taken to serve as the basis (once divided by an uncertainty factor of 3) for the EPA's acute NOAEL (14 mg/kg) and acute RfD (0.14 mg/kg) for imidacloprid (U.S. EPA/OPP 2003).

A study investigating the chronic toxicity and carcinogenicity of imidacloprid in rats (Eiben and Kaliner 1991; Eiben 1991) (Appendix 2) serves as the basis for the EPA's Chronic RfD of 0.057 mg/kg/day for imidacloprid (U.S. EPA/OPP 2003). The critical effects seen in this study were depression in body weight gain (both sexes) and mineralization of the colloid of the thyroid follicles (both sexes, but males affected at a lower dose), yielding a chronic NOAEL of 5.7 mg/kg body weight/day (100 ppm diet).

4.1.2.2. Birds – The available studies of imidacloprid toxicity in birds (Appendix 3) are standard studies (e.g. EPA guideline studies) on quail and duck submitted to EPA under pesticide registration requirements, as well as non-standard studies (open literature) on songbirds; an overview of avian toxicity values derived from these studies is shown in Table 4-1. On the basis of acute oral toxicity, house sparrows and Japanese quail appear to be the most sensitive species, with NOAELs of 3 and 3.1 mg a.i. imidacloprid /kg body weight, respectively. On the basis of chronic dietary reproduction studies, bobwhite quail was the most sensitive species, with an LOAEC of 36 ppm a.i. imidacloprid in the diet for significantly reduced hatchling body weight. Using data on body weight and food consumption from the Toll (1991b) study, it is possible to

convert this value to an LOAEL of 2.5 mg imidacloprid/kg body weight/day. Mallard ducks appear to be the most tolerant avian species tested; a chronic NOAEC of 128 ppm a.i. imidacloprid in the diet was determined on the basis of reproduction, growth and survival (Toll 1991c; Stafford 1992; Hancock 1994b).

A field study conducted using Merit 0.62% Granular formulation examined the survival and mortality of common species of birds found at eight different golf courses. There were no statistically significant differences in survival among banded birds assessed by visual and radio telemetry on treated (0.5 lb a.i./acre) and un-treated plots (Toll and Fischer 1993).

Several studies conducted with red-winged blackbirds (Avery et al. 1993a,b), ringed turtle doves and house sparrows (Hancock 1994) demonstrated that birds learn to avoid imidacloprid-treated seed, especially when the treated seed is clearly identifiable (e.g., bright red). The learning is hypothesized to take place through post-digestive distress and subsequent avoidance.

4.1.2.3. Terrestrial Invertebrates –The body of literature which discusses the efficacy, mechanism of action and potential harmful effects of imidacloprid on terrestrial invertebrates is large and complex. There are numerous studies on the efficacy of imidacloprid in controlling pests in agricultural crops, decorative plants and animals of economic interest. Similarly, there are many studies which address the mechanism of action of imidacloprid in controlling insects, and the development of resistance to imidacloprid. Summarizing all of these studies in detail is well beyond the scope of this document. Given the overall purpose of this investigation in determining potential hazards of imidacloprid to humans and ecological receptors of interest, this section focuses on the potential adverse impacts of imidacloprid on beneficial arthropods and other terrestrial invertebrates. These studies are summarized in Appendix 4. An overview of the key studies, and toxicity values derived thereof, is presented Table 4-2 for bees and earthworms, and Table 4-3 for predatory arthropods. Given that imidacloprid is a neurotoxic insecticide, it is no surprise that honey bees and parasitic wasps are among the most sensitive species tested.

Imidacloprid is a systemic insecticide used on plants (via soil application, foliar application or seed dressing) to control insects with sucking or piercing mouthparts, including rice hoppers, aphids, thrips, whiteflies, termites, turf insects, soil insects and certain beetles. The U.S. Forest Service uses imidacloprid primarily to control Hemlock woolly adelgid (*Adelges tsugae Annand*) infestations.

Imidacloprid is also applied to the skin of dogs and cats to control fleas (imidacloprid and permethrin are the active ingredients in K9-Advantix® which is effective against mosquitos, fleas and ticks; imidacloprid is the active ingredient in Advantage®, which is only effective against fleas). It is of interest to note that topically applied imidacloprid spreads out in the superficial lipid layer of the skin, where it remains effective until the dog sheds that layer. Systemic absorption of imidacloprid is irrelevant to its efficacy in killing fleas. The fleas are killed upon contact with the pet dander, and don't need to bite the pet. Fleas exposed to dogs previously treated with imidacloprid, but whose fur had been cleansed of all active material, were not killed. Bayer reports that fleas exposed to shaved hairs from imidacloprid-treated dogs

had symptoms (tremor, immobilization, and death) similar to the fleas exposed to the dog itself – see http://www.animalhealth.bayerhealthcare.comAdvantage_Application.

The mechanism of action of imidacloprid in insects has been extensively studied, and is well known. In essence, imidacloprid activates nicotinic acetylcholinesterase receptors (nAChR) though binding at or near the sites where nicotine and acetylcholinesterase bind, resulting in dysfunction of the nervous system, immobilization and death (see Section 3.1.2 for details). In general, effects on the nervous system are seen very quickly after exposure, whereas mortality develops 4 or more hours later. Both ingestion and contact routes of exposure are effective in controlling insect pests. Insects generally cease feeding activity upon exposure and most are killed within 4 hours to seven days of exposure. Studies have shown that insects are capable of developing resistance to imidacloprid, but do so with lower magnitude and less rapidity than with other insecticides (Zewen et al. 2003; Liu et al. 2004; Devine et al. 1996).

Bees

There is an ongoing debate as to whether the use of imidacloprid has an adverse effect on honey bees under field conditions. This is due in large part to an unfortunate outbreak of a "novel bee malady" in Central and Western France in 1996. Since imidacloprid, marketed as Gaucho®, recently (1994) had become used widely as a seed dressing for sunflowers, some beekeepers assumed that it was responsible for the malady. Although Gaucho® has been suspended from use in France since 1997, the bee malady apparently continues (Schmuck et al. 2001).

As a consequence of the above misfortune in France, the impact of imidacloprid and its metabolites on honey bees has been well studied in both laboratory and field tests. The major studies are summarized in detail in Appendix 4. In short, laboratory studies demonstrate that imidacloprid is acutely toxic to bees at low doses, (48-hour LD_{50} values ranging from 3.7 to 230.3 ng/bee) and has sub-lethal effects on behavior and the insect nervous system at even lower doses (e.g., 0.1 ng/bee). However, the consequences of the behavioral toxicity demonstrated under laboratory conditions, particularly the conditioned reflexes used as a gauge of learning, and possibly foraging behavior, remain to be elucidated with respect to their impacts on populations in the field. Chronic studies, conducted with bees from different countries and tested in different seasons, fail to demonstrate any adverse effects on foraging activity, mortality or colony vitality, at dietary concentrations of approximately 20 to 24 ug imidacloprid per kg sucrose or honey (equivalent to approximately 1 ng/bee). These studies are discussed in more detail as follows.

Acute oral 48-hour LD_{50} values range from 3.7 to 81 ng/bee, and contact 48-hour LD_{50} values range from 8 to 230.3 ng/bee. The combined studies of Cole (1990), Nauen et al.(2001) and Schmuck et al.(2001) indicate that the NOAEL for acute mortality is approximately 1.2 ng a.i./bee. Assuming a body weight of 0.000093 kg/bee, this is equivalent to a dose of 0.013 mg/kg body weight.

Imidacloprid formulations yielded 48-hour LD_{50} values in bees in the same range as for technical grade imidacloprid. Acute toxicity varies widely among the different imidacloprid metabolites (Appendix 4). The olefin, hydroxy- and dihidroxy-metabolites are of the same order of acute

toxicity as imidacloprid. The 6-chloronicotinic acid, urea- and desnitro- metabolites are essentially non-toxic with 48-hour LD_{50} values ranging from about 1000 ng/bee to over121,500 ng/bee.

The timing of the onset of toxicity (behavioral or nervous system effects) versus onset of mortality has been studied (Suchail et al. 2001, 2004; Moncharmont et al. 2003). The available studies suggest that while imidacloprid is responsible for initial toxicity, some of its primary metabolites (olefin and 5-hydroxyimidacloprid) may be responsible for delayed mortality. Further evidence in support of this comes from electrophysiological studies; binding studies and pharmacokinetic studies (Nauen et al. 2001; Suchail et al. 2001, 2004). In bees and other insects, signs of toxicity (e.g., immobilization) are seen almost immediately following exposure, while the onset of mortality is generally 4 hours after exposure. Pharmacokinetic studies with honey bees show that primarily unchanged imidacloprid is present during the first twenty minutes of oral or contact exposure, during which the initial toxic effects, but not mortality, are observed. The appearance of the olefin- and 5-hydroxy- metabolites as major systemic residues occurs in correspondence with the onset of mortality, suggesting that these metabolites, or some other unidentified residues, rather than unchanged imidacloprid, are responsible for mortality (Suchail et al. 2001, 2004).

Behavioral and electro-physiological studies with honey bees and other insects have demonstrated effects of sublethal concentrations of imidacloprid on learning, conditioned responses, and the nervous system. In many cases, the dose-response is anomalous, with extremely low doses causing either a promotion or inhibition of the endpoint of interest, and higher doses causing the opposite response (Lambin et al. 2001; Armengaud et al. 2000; Guez et al. 2001; Matsuda et al. 2001, Zafeiridou and Theophilidis 2004). For example, in the study by Lambin et al. (2001), imidacloprid at a concentration of 1.25 ng/bee reduced the habituation of the proboscis extension reflex (imidacloprid-exposed bees were better at learning than controls) and increased motor activity relative to controls. However, higher concentrations (2.5 to 20 ng/bee) caused dose-related impairment of activity and increased the gustatory threshold for learning. Investigators hypothesized that the anomalous dose-response results from these and other studies support the existence of multiple nAChR binding sites for imidacloprid.

Laboratory studies investigating the effects of bee age and season of the year on response to imidacloprid exposure are equivocal. Following approximately 10 days of exposure to imidacloprid, Decourtye et al. (2003) found that bees collected in winter were more sensitive to the effects of imidacloprid on mortality, but bees collected in summer were more sensitive to effects of imidacloprid on behavior and conditioned responses. Guez et al. (2001) demonstrated significant differences in the timing and dose-response of habituation of a conditioned reflexive response (proboscis extension reflex) in 7-day old versus 8-day old bees. However, using bees from seven different apiaries from 5 countries, Nauen et al.(2001) did not demonstrate any effect of season, age or location on acute mortality measured in terms of 48-hour LD_{50}.

There is some question whether bees develop avoidance behavior toward imidacloprid. Field studies using label-recommended rates and procedures suggest that foraging activity is affected only when granular imidacloprid is applied without recommended irrigation (Gels et al. 2002).

Laboratory studies suggest that imidacloprid-treated sucrose consumption may be dependent on concentration. Nauen et al. (2001) found that honey bees rejected sucrose solutions containing imidacloprid at concentrations of 1 mg/kg or higher. However, in studies where bees were exposed to low sub-lethal concentrations (4 and 8 ug/L), there was no difference in food consumption between controls and imidacloprid-exposed bees (Dechaume Moncharmont et al. 2003). It is not clear whether the observed imidacloprid-related decreases in food consumption observed by Nauen et al (2001) are due to avoidance or to a knockdown effect (bees immobilized and unable to feed).

Schmuck et al (2001) conducted a series of studies to demonstrate whether imidacloprid-treated sunflower seeds or sunflowers grown in fields previously treated with imidacloprid could adversely affect honey bees. These investigators exposed honey bees to imidacloprid-doped sunflower honey at concentrations up to 0.020 mg/kg (selected on the basis of residue studies with sunflowers) for 39 days. No mortality or adverse effects on feeding activity, wax/comb production, breeding or colony vitality were detected at any concentration, yielding an NOAEC of 0.020 mg imidacloprid/kg honey. It is not possible to convert this dietary concentration to a dose, because foraging activity was measured in terms of total honey and pollen accumulated over the 39-day experimental period (i.e., it is not possible to estimate consumption per bee from the data given). There were no differences in total honey or pollen collection between controls or bees exposed to imidacloprid at any concentration. In addition, Schmuck et al. (2001) determined that sunflowers either grown from imidacloprid-dressed seed, or grown on imidacloprid-treated soil (label application rates, 3-4 different fields in two locations in Germany) had no detectable residues of imidacloprid (detection limit = 0.0015 mg/kg) in the pollen or nectar. Consequently, the investigators concluded: “From these findings it is evident that honeybees are not exposed to residues of imidacloprid or structurally related imidacloprid metabolites when foraging on sunflower plants, irrespective of whether these plants have been cultivated on previously imidacloprid-treated soils or had been raised from imidacloprid-dressed seed.” They state further: “This conclusion is supported by the fact that no impacts such as depopulation of hives, immobilized or disorientated bees or increased mortality could be observed in several tunnel and field studies on imidacloprid-treated sunflowers” (Schmuck 1999 as cited in Schmuck et al. 2001).

A residue study conducted by Laurent and Rathahao (2003) confirms that imidacloprid residues do not accumulate to any significant extent in sunflower pollen. In this study, sunflowers were grown under field conditions from seed dressed with imidacloprid at a rate of 1 mg imidacloprid per seed. Imidacloprid residues ranging from not detected to 36 ng/g (equivalent to 36 ug/kg), with a mean (± SD) of 13±13 ng/g (equivalent to 13 ± 13 ug/kg), were detected in pollen (detection limit = 0.5 ng/g or 0.5 ug/g). In comparing their study with the findings of Schmuck et al. (2001), Laurent and Rathahao (2003) noted that they dressed their seed with 30% higher than the recommended label rate (1 mg a.i./seed, versus 0.7 mg a.i./seed recommended). Schmuck et al. (2001), who failed to detect imidacloprid residues in pollen or honey, used the recommended label rate. Neither investigator reports the percentage organic content in the soil in which their plants were grown. Differences in soil organic content can result in differences in imidacloprid uptake, with higher uptake observed in plants grown from imidacloprid-dressed seeds in soil with higher organic content (Rouchaud et al. 1994).

Taken together, the laboratory studies which investigated longer-term exposure to imidacloprid yield a chronic NOAEC of approximately 1 ng/bee (Decourtye 2003; Schmuck 2001; Dechaume Moncharmont 2003; Decourtye et al. 2004). Studies that investigated sub-lethal behavioral effects, such as proboscis extension reflex (PER) and olfactory learning, suggest that the chronic NOAEC may be lower (on the order of 6 ug/kg dietary concentration or approximately 0.24 ng/bee), but the relevance of these studies to actual foraging activity in the field is uncertain (Lambin 2001; Guez et al. 2001; Decourtye et al. 2003); Decourtye et al. (2004) demonstrated there was no significant difference in foraging (measured by sucrose consumption) between controls and imidacloprid-exposed bees at imidacloprid concentrations as high as 24 ug/kg diet (equivalent to approximately 1 ng/bee/day).

Field studies conducted in Kentucky with granular formulations of imidacloprid (Merit) showed no adverse effects on bumble bees under field conditions. There were no adverse effects on colony vitality or honey production among bumble bee colonies caged on imidacloprid-treated plots with flowering white clover (Merit 0.5 Granular, applied at maximum label rate for white grubs [0.4483 kg a.i./ha], with irrigation) with respect to untreated control plots. Gels et al. (2002) also conducted a similar field study to assess the effect of irrigation versus non-irrigation on caged bumble bees foraging on turf plots treated with Merit® 75 (0.336 kg a.i./ha). With respect to bees foraging on untreated plots, there was no effect on colony vitality or worker bee defensive response on imidacloprid-treated plots which were irrigated following application. However, fewer honey pots and brood chambers, fewer workers, reduced biomass of workers and reduced colony weight were observed among bees foraging on imidacloprid-treated plots which were not irrigated.

A study conducted in support of pesticide registration with EPA investigated the pattern of mortality among caged honey bees exposed to imidacloprid-treated foliage (Hancock et al. 1992). Mortality was assessed 2, 8, and 24 hours after bees were caged with imidacloprid-treated alfalfa (0.045, 0.167 and 0.5 lb a.i./acre), and the residual time needed to reduce chemical activity such that bee mortality was less than 25% (RT_{25}) was calculated (smaller numbers are better). The estimated RT_{25} values are: <2 hours, < 8 hours, and 8 hours, for application rates of 0.045, 0.167, and 0.5 lb a.i./acre, respectively. The RT_{25} of < 2 hours for 0.045 lb a.i./acre indicates that imidacloprid may be applied at this rate with minimal hazard to bees during early morning, or late in the evening when bees are not actively foraging. The RT_{25} < 8 hours associated with 0.167 lb a.i./acre indicates that imidacloprid may be applied at this rate with minimal hazard to bees late in the evening when bees are not actively foraging. The RT_{25} = 8 hours associated with 0.5 lb a.i./acre indicates that imidacloprid may be applied at this rate with moderate hazard to bees late in the evening when bees are not actively foraging.

Beneficial Predatory Arthropods

The effects of imidacloprid have been studied on terrestrial invertebrates (mostly insects) that are used as predators in integrated pest management systems. Most of these studies were conducted with imidacloprid formulations applied in laboratory or field-like settings to approximate the recommended field application rates, and with endpoints such as acute mortality, longer-term mortality, fecundity, susceptibility to predators, and ability to infect prey. The results of these studies are mixed, with imidacloprid causing harm, causing no harm, or

enhancing the fecundity or predatory function of the predator under study. The main studies located in the open literature are summarized in Appendix 4. An overview of the toxicity values derived from these studies, tabulated in terms of formulation, is given in Table 4-3.

Large-scale field studies conducted with commercially available formulations of imidacloprid (Merit 75 wettable powder, Merit 0.5% granular) found no adverse impacts on the abundance of soil micro-arthropods or beneficial predators, when applied to turf-grass at label application rates (Kunkel et al. 1999; Zenger and Gibb 2001)

Imidacloprid has been shown to act synergistically with parasitic entomopathogenic fungi, nematodes and beetles in controlling insect pests (Quintela and Mccoy 1997; Kaakeh et al. 1997, Koppenhofer and Kayla 1998).

Some studies suggest that imidacloprid increases the fecundity of beneficial mite populations. James (1997) demonstrated that application of Confidor 350 SC to control aphids in an apricot orchard in Australia (applied to runoff via air-blast sprayer, 15 ml per 100 L, 0.0053% imidacloprid) significantly reduced the population of *Amblyseius victoriensis* (beneficial phytoseiid mite) 4 weeks following application. However, the population recovered at 5-6 weeks following application, and was more than twice the size of the untreated control population (in another area of the orchard) by 9-12 weeks post-application. Imidacloprid was also shown to increase the fecundity of the Two-spotted spider mite, *Tetranychus urticae*, in hop fields sprayed with imidacloprid for purposes of controlling the hop aphid, *Phorodon humuli* (James and Price 2002).

In some of the insects which were adversely affected in short-term studies, the symptoms and patterns of toxicity and mortality were similar to those observed in honey bees, with significant early intoxication (immobilization) followed by delayed mortality (Hewa-Kapuge et al. 2002; Kunkel et al. 2001; James 1997; Grafton-Cardwell and Gu 2003). In some cases, although there was initial toxicity among 100% of the test organisms, complete recovery was observed in the majority of organisms within several days.

Information from the available studies suggests that the method of imidacloprid application may be important in determining whether or not a short-term hazard is likely to be incurred by a predatory arthropod. In some studies with sensitive species, direct contact with a sprayed formulation resulted in either neurotoxicity or mortality, but exposure via ingestion of residues in soil or on plant foliage were not as harmful (Brunner et al. 2001; Hewa-Kapunge et al. 2003; Brunner et al. 2001; Delbecke et al. 1997; James 1997). Likewise, soil application of either sprayed or granular products followed by irrigation was not harmful, whereas application without irrigation led to adverse effects on the experimental species under observation. However, as Grafton-Cardwell and Gu (2003) demonstrated with the Vedalia beetle, the above observations do not always hold true. In these studies, exposure of Vedalia beetles to their prey (cottony cushiony scale larvae) which had been raised either on plants growing in imidacloprid-treated soil or sprayed to runoff with imidacloprid formulation, resulted initially in significantly reduced mean percentages of survival among adult beetles and their progeny, with respect to unexposed controls. In spite of the initial transient mortality, imidacloprid-exposed Vedalia

beetle populations rebounded to equal control numbers within 43 to169 days post-exposure, depending on whether one considers adult or larval survival variables (details in Appendix 4).

The repeated observation that imidacloprid-exposed insect populations rebound after initially observed increased mortality or reduced fecundity (Hewa-Kapuge et al. 2002; Kunkel et al. 2001; James 1997; Grafton-Cardwell and Gu 2003) deserves additional consideration. It calls into question the validity of using the results of short-term laboratory studies (LD_{50} studies, for example) to determine whether or not the use of imidacloprid under field conditions causes adverse effects on populations.

Walthall and Stark (1997a,b) addressed the above consideration in a study with pea aphids which was designed to determine whether the acute 72-hour LC_{50} was a good predictor of the effects of a pesticide on a population. To do this, they conducted an acute toxicity study and compared it to the results of a life table study in which exposed individuals were monitored from birth through adulthood (mortality and reproduction were recorded every 24 hours for each aphid). In the acute study, potted broad bean plants (*Vicia faba L.*) were sprayed with an imidacloprid formulation (240FS, 240 g a.i./L) at one of eight concentrations (control, 0.1, 0.175, 0.25, 0.35, 0.5, 0.7 and 1.0 mg/l), then infested with Pea aphids (*Acyrthosiphon pisum Harris*). The acute LC_{50} values were determined for both adult and neonate aphids at 24-hour intervals; 72-hour LC_{50} values for neonates and adults were 0.225 mg/L and 0.468 mg/L, respectively. Based on results of the acute study, the chronic life table study used a control and eight concentrations ranging from 0 to1.25 mg/L imidacloprid. The chronic study determined net reproductive rate (R_o), the intrinsic rate of increase (r_m) and realized fecundity (U_x) for the imidacloprid-exposed and unexposed populations. The authors concluded:

> *"An examination of R_o indicated that sublethal effects were occurring that reduced reproduction. However, by looking at the mean number of offspring produced per surviving female and U_x, it was determined that the reduction in R_o was entirely due to acute mortality and a reduction in life span. Also, exposure to increasing concentrations of imidacloprid did not cause a shift in either the day of initial reproduction or the day of peak reproduction. Therefore, this pesticide caused no sublethal effects on reproduction and, as such, a lethal concentration estimate should have been a good predictor of effect at the population level. However, the 72-hour lethal concentration estimate was not a good predictor of effect of this pesticide on population growth. Populations exposed to the 72-hour LC_{50} were able to maintain rates of population increase (r_m = 0.224) similar to those of the control (r_m = 0.295). The data indicate that the reason for the discrepancy between acute lethal concentration estimates and population growth was that surviving individuals were able to sustain heightened rates of reproduction following acute exposure to imidacloprid. The ability of surviving individuals to maintain these high reproductive rates allowed them to compensate for losses and act as reservoirs for future reproduction. It is not possible, using acute mortality estimates alone, to predict this "reservoir effect", and therefore not possible to predict how a populations growth rate will respond or change based on this endpoint. Thus this would suggest that the assessment of a xenobiotic based solely on acute mortality estimates will lead to flawed conclusions about a populations exposure response."*

Worms

Zhang et al. (2000) and Luo et al. (1999) determined LC_{50} values for the earthworm, *Eisenia foetida* when exposure to imidacloprid was tested by immersing worms in imidacloprid solutions

(48-hour LC_{50} = 0.77 mg/L), placing them on imidacloprid-treated filter paper (48-hour LC_{50} = 0.034 ug/cm^2) or placing them in artificial soil (7-day and 14-day LC_{50} values = 3.48 and 2.30 mg/kg dry soil, respectively). Laboratory studies with earthworms demonstrated that imidacloprid exposure could cause DNA damage and a dose-related increase in sperm deformity (Luo 1999; Zhang et al. 2000), with a NOAEC for sperm deformity of 0.1 mg/kg dry soil and a LOAEL of 0.5 mg/kg soil.

In a series of large field tests on turf-grass in Kentucky, Merit 75 wettable powder and Merit 0.5G caused a short-term reduction of earthworm abundance during fall application, but only Merit 0.5G caused a transient reduction in abundance in spring. However, earthworm abundance was no different from that of untreated control plots in either season 36 - 40 days after treatment (Kunkel et al. 1999).

Mostert et al. (2000) tested imidacloprid on *Pheretima* group earthworms commonly found in South African turf-grass, and found acute LC_{50} values (7-day LC_{50} = 3.0 mg/kg soil or 15.8 mg/0.1m^2) to be higher than the label-specified maximum application rate of imidacloprid on turf-grass (1000 ml/ha or 0.35 kg a.i./ha or 3.5 mg/0.1m^2).

Interactions with Biological Control Agents

Some efficacy studies on imidacloprid have suggested a synergistic effect with biological control agents. Imidacloprid was shown to act synergistically with entomopathogenic fungi to kill first instars of the root weevil *Diaprepes abbreviatus* at doses between 100 and 1000 ppm (Quintela and Mccoy 1997). There was no difference in efficacy between oral and contact routes of exposure to imidacloprid. While imidacloprid alone was ineffective in killing cockroaches (they recovered after initial incapacitation), the combination of *Metarhizium anisopliae* (Imperfect Fungi: Hyphomycetes) and imidacloprid was effective, suggesting possible synergistic activity (Kaakeh et al. 1997). Imidacloprid had no significant positive or negative impact on the efficacy of a nucleopolyherovirus in controlling tobacco budworm *Heliothis virescens* (Koppenhofer and Kaya 2000). Similarly, imidacloprid was shown to have no adverse impacts on entomopathogenic nematodes used to control moths, cutworms or white grubs (Zhang et al., 1994; Koppenhofer and Kaya 1998).

4.1.2.4. Terrestrial Plants (Macrophytes) – Anecdotal and undocumented reports of phytotoxicity are made in some general review articles on imidacloprid. However, two published studies, one conducted with hops, and one conducted with Eastern Hemlock, failed to note phytotoxicity following label-recommended application methods and rates. The study with hops employed foliar application methods, with phytotoxicity only evident when Amulsol or GPC100 were used as additives, but not when imidacloprid was used alone (Weichel and Nauen 2004). In the study with Eastern Hemlock (Webb et al. 2003) imidacloprid was applied via soil drench (Merit 75WP, using the highest labeled application rate of 2 g product per 0.95 L solution per 2.5 cm dbh). Following treatment and the removal of adelgids, infested trees "recovered dramatically" with new growth. Field or laboratory studies which address standard measures of plant growth and survival (e.g., vegetative vigor or seedling emergence) following treatment with imidacloprid were not found in the available literature. Westwood et al. 1988 reported no

meaningful difference in emergence between controls (88%) and sugar beets grown from imidacloprid-dressed seed (84%; 0.7 mg a.i. imidacloprid/seed).

Imidacloprid absorption and translocation has been studied in plants following trunk injection (Tattar et al. 1998), soil injection (Tattar et al. 1998), seed application (Rouchaud et al. 1994; Westwood et al. 1998; Laurent and Rathahao 2003; Schmuck et al. 2001) and foliar application (Weichel and Nauen 2004; Bucholz and Nauen 2002). In general, imidacloprid is rapidly absorbed and transported to the foliage (i.e. above-ground portions) of the plant, with very little found in the roots. In cases where imidacloprid is applied to soil, and the concentration of imidacloprid and imidacloprid residues are studied as the plant grows, increasingly smaller quantities of imidacloprid and imidacloprid residues are found in successively newer growing portions of the plant (i.e. heart leaves, flowers). The highest concentrations are found in the oldest parts of the plants: cotyledons and older leaves. Unchanged imidacloprid is found in the highest concentration, but the olefinic, guanidine, hydroxy- ,5-hydroxy- , urea- and metabolites also have been detected in smaller quantities. In sugar beets grown from imidacloprid-dressed seed, Westwood et al. (1998) determined that imidacloprid concentrations were highest in young plants 21 to 49 days old (1.2-15.2 ug imidacloprid/g fresh plant weight), while the olefinic metabolite reached it's peak concentration in the foliage of older plants, 67 to 97 days old (0.3 - 0.43 ug olefinic metabolite/kg fresh plant weight).

In a preliminary study using Merit 75 WP (soil injection: 1.25 g a.i/inch diameter at breast height [dbh], at 2 gallons per inch dbh) and Mauget capsules (tree injection: 3 ml of 15% imidacloprid each capsule at number to give 0.225 g a.i./inch dbh), Tattar et al. (1998) determined that trunk injection was more effective than soil injection in producing more rapid and higher concentrations of imidacloprid in the foliage of Eastern Hemlock trees. Peak imidacloprid concentrations were detected in Eastern Hemlock foliage 4 to 8 weeks after trunk injection (7.9 ppm foliage, remaining between 0.5 and 1 ppm through 20 weeks post-injection), but not peaking above the label-listed efficacy threshold (for sucking pests) of 0.15 ppm until 12 - 20 weeks after soil injection (approximately 0.5 ppm). This trend was also observed in pin oak. However, in white pine, trunk injection (0.15 ppm peaking at 20 weeks post-injection) was less successful than soil injection (approximately 0.5 ppm peaking at 12 weeks post-injection, then declining to approximately 0.15 ppm by 20 weeks post-injection).

Stewart and Stewart (1995) determined that imidacloprid in a 5% ready-to-use trunk spray treatment (trunk below 4.5 feet sprayed thoroughly) was effective in controlling hemlock woolly adelgid only in trees with DBH less than 7 inches. This suggests that older, larger trees may not absorb enough imidacloprid through the trunk to be effective.

4.1.2.5. Terrestrial Microorganisms – Using measurements of hydrolysis, photolysis and soil respiration, Liu et al. (2001) report that imidacloprid (up to 0.100 mg/L) and its metabolites (up to 0.04 mg/L) had little effect on soil microorganisms. This is an abstract of a study written in Chinese, and as such, no other details are readily available.

Imidacloprid applied to sandy soil at a rate of 10 mg a.i/kg soil was shown to inhibit fungal growth but not bacterial growth with respect to untreated control soil, after 2-weeks of incubation in laboratory conditions (Tu 1995).

4.1.3. Aquatic Organisms

4.1.3.1. Fish – The acute and chronic toxicity of imidacloprid to fish has been studied in standard laboratory species. A summary of the available studies is presented in Appendix 5. For freshwater species, static 96-hour acute LC_{50} values ranged from > 105 mg a.i./L for bluegill (Bowman and Bucksath 1990a) to 211 mg a.i./L for rainbow trout (Grau 1988a). A test with a saltwater species, sheepshead minnow, yielded a 96-hour acute LC_{50} value of 161 mg a.i./L (Ward 1990a). Using the standard classification scheme proposed by U.S. EPA/EFED (2001), imidacloprid would be classified as *practically nontoxic* to fish.

A 98-day flow-through early life stage test was conducted with rainbow trout in response to EPA's requirements for testing as part of the pesticide registration process (Cohle and Bucksath 1991; Gagliano 1992). No statistically significant or biologically important effects of imidacloprid exposure were observed with respect to egg viability, hatch, survival or behavioral variables. The most sensitive endpoint was a significant reduction in body length at 36 and 60 days post-hatch. The NOAEC for this endpoint was 9.8 mg/L. Based on a re-analysis (Gagliano 1992) of the Cohle and Bucksath (1991) data for day-36 post-hatch body length, this study yields an NOAEC of 1.2 mg a.i./L and a LOAEC of 2.3 mg a.i./L. This effect, however, was not seen at 60 days post-hatch.

4.1.3.2. Amphibians – Two studies are available which assess the toxicity of imidacloprid to amphibians. These studies are included with the data on fish in Appendix 5.

In a study published in the open literature, Feng et al. (2004) determined 96-hour LC_{50} values of 82 and 129 mg/l for technical grade imidacloprid (> 95% active ingredient) in tadpoles of *Rana linocharis* and *Rana hallowell*, respectively. The NOAEC values for these species were 16.7 and 67.5 mg/l, respectively. Unpublished LC_{50} values of 176 to 220 mg/L are reported for Ranids by Julian and Howard (1999) in their MRID study of the effects of three insecticides, including imidacloprid, on the hatching and development of four species of amphibians.

Based on results from *in vitro* studies with erythrocytes from tadpoles, Feng et al. (2004) suggest that imidacloprid may cause chromosomal and DNA damage at sub-lethal concentrations (NOAEC = 2 mg/L in tadpole micronucleus test; LOAEC = 0.05 mg/L in Commet Assay of DNA damage).

However, Julian and Howard (1999) failed to identify statistically significant effects of imidacloprid exposure on hatching success or percentages of malformations in *in vivo* tests with four different species of amphibians. Imidacloprid had no effects on hatching success of *Rana pipiens*, *Pseudacris triseriata*, *Ambystoma jeffersonianum*, or *Bufo americanus*, tested at imidacloprid concentrations ranging from 1.75 mg/l to 110 mg/l, in comparison with controls. Similarly, there were no statistically significant differences among treatments with respect to hatchling deformities. However, the most sensitive species, *P. triseriata* tadpoles exposed as

egg masses to the highest imidacloprid concentration tested (88-110 mg/L) had approximately 24% (23-25%) total hatchling deformities, with respect to 11.2% (2.5 -15%) for controls. It may be that the high variability in the control tadpoles resulted in lack of significance. The other species tested had control percentages of total deformities ranging from 3.9% to 10.5%.

4.1.3.3. Aquatic Invertebrates – Standard laboratory studies on freshwater and saltwater species, as well as a microcosm study have been conducted with technical grade imidacloprid. A summary of the available studies on aquatic invertebrates is presented in Appendix 6 and the key toxicity values from these studies are summarized in Table 4-4. On the basis of both acute and chronic toxicity, crustaceans and aquatic insects are more sensitive to imidacloprid than fish.

Amphipod crustaceans such as *Hyalella azteca*, the saltwater Mysid, *Mysidopsis bahia*, and the fresh water insect midge, *Chironomus tentans*, are the most sensitive species. In freshwater, the water flea, *Daphnia magna*, was the least sensitive species, while in saltwater, the eastern oyster was least sensitive. An overview of the relevant acute and chronic toxicity values from the available studies are shown in Table 4-2 for comparison. Acute toxicity values range from a 96-hour NOAEC of 0.000035 mg/L for *H. azteca* (England and Bucksath 1991), to a 96-hour NOAEC of 145 mg/L for eastern oyster (Wheat and Ward 1991). On the basis of longer-term studies designed to assess reproduction, growth and survival, *M. bahia* was the most sensitive species, with an NOAEC value of 0.000163 mg a.i. imidacloprid/L for growth and reproductive success (Ward 1991), and *D. magna* was the most tolerant species with a 21-day NOAEC for immobility of 1.8 mg/L (Young and Blake 1990).

A 19-week microcosm study (Appendix 6) conducted as part of EPA's pesticide registration requirements for imidacloprid confirms the results of the above laboratory studies (Moring et al. 1992). Technical grade imidacloprid was applied to the surface of tanks containing a variety of phytoplankton, zooplankton, and macro-invertebrates at two week intervals, for a total of 4 applications. Concentrations ranging from 0 to 0.180 mg a.i./L were employed. Amphipods were determined to be the most sensitive species, with statistically significant impacts on abundance at some sampling intervals at the lowest concentration tested, yielding an LOAEC of 0.002 mg a.i./L. Statistically significant decreases in populations of total macro-invertebrates as well as individual macro-invertebrate taxa (mayfly, midge, caddisfly, beetle and amphipod) were most frequently observed (at different sampling endpoints) at imidacloprid concentrations ranging from 0.02 to 0.180 mg a.i./L. On the basis of these findings, the study authors recommended 0.006 mg a.i./L as a regulatory NOAEC for imidacloprid in aquatic systems. However, the results of previously discussed laboratory studies (Gagliano 1991; Ward 1991), as well as the results for amphipods at some sampling intervals in this study, suggest that the NOAEC for growth and survival of sensitive macroinvertebrate species is on the order of 0.000163 mg a.i./L.

None of the imidacloprid metabolites tested (urea metabolite NTN 33519; 6-chloronicotinic acid and NTN 33823) were as acutely toxic as technical grade imidacloprid in tests with the midge (*C. tetrans*) or amphipod (*H. azteca*) (Bowers1996a; Bowers and Lam 1988; Rooney and Bowers 1996; Dobbs and Frank 1996b). In tests with *M. bahia*, a formulation of imidacloprid, NTN

33893 240 FS, had the same order of acute toxicity as technical grade imidacloprid (Lintott 1992).

4.1.3.4. Aquatic Plants – The available studies on the toxicity of imidacloprid to aquatic plants are given in Appendix 7. The acute toxicity of imidacloprid was tested on green algae as part of EPA's pesticide registration process (Heimbach 1989; Gagliano and Bowers 1991). These studies yielded NOAEC values for biomass and growth equivalent to the limits of the tests (i.e., 119 mg a.i./L for 5-day test with *Selanastrum capricornutum*; 10 mg a.i./L for *Scenedesmus subspicatus*).

A 4-day NOAEC of 6.69 mg a.i./L was determined for the diatom (*Navicula pelliculosa*) following exposure to a 21.6% imidacloprid formulation (Hall 1996).

Statistically significant decreases of cyanophyte populations (blue-green algae) were observed at concentrations of 0.020 mg/L and higher at some sampling points in the microcosm study of Moring et al. (1992). However, a laboratory study on blue-green algae in support of pesticide registration (Bowers et al. 1996b) does not support the biological significance of the transient effects observed by Moring et al. (1992). On the basis of biomass and growth, Bowers et al (1996b) report 4-day EC_{25} and EC_{50} values of 26.7 and 32.8 mg a.i./L, respectively, with a 4-day NOAEC of 24.9 mg a.i./L.

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview

As in the human health risk assessment and for the same reasons, the quantitative exposure assessments are detailed in four EXCEL workbooks by application method and soil type:

broadcast applications of liquid formulations on clay or loam soils;
broadcast applications of granular formulations on clay or loam soils;
soil injections in clay or loam soils;
applications (any method) to predominantly sand soils.

While this approach is more complicated than that taken in most Forest Service risk assessments, it is necessary because exposures vary substantially with the different application methods for imidacloprid. For tree injection, no quantitative exposures are presented. For the same rationale articulated in the human health risk assessment, there is no basis for asserting that substantial exposures to most terrestrial organisms are plausible from tree injection. A major exception, of course, is the target species (adelgids) and other insects that might feed on treated trees. Additional and perhaps significant exposures are likely to some beneficial insects that prey on adelgids and other insect pests of hemlocks. Potential risks to these species are characterized using the available field or field simulation studies summarized in Section 4.3.2.3 (Dose-Response Assessment for Terrestrial Invertebrates).

For soil injection applications as well as broadcast applications, exposures to soil organisms are likely and these exposures are discussed below in Section 4.2.4. Exposures to other terrestrial animals from soil injection will primarily involve contaminated water. These exposures are summarized in Attachment 3 for applications to loam or clay soils and Attachment 4 for applications to predominantly sandy soils. As discussed in Section 3.2.3.4.6, the estimated concentrations of imidacloprid in surface water are similar for sandy soils after applications by broadcast or soil injection. Thus, all of these application methods are covered for sandy soils in Attachment 4.

While the Forest Service does not anticipate using broadcast applications of liquid or granular formulations, these application methods are covered in the current risk assessment. For broadcast applications, terrestrial animals might be exposed to any applied pesticide from direct spray, the ingestion of contaminated media (vegetation, prey species, or water), grooming activities, or indirect contact with contaminated vegetation. As with the human health exposure assessment, two sets of exposure scenarios are provided in two separate EXCEL workbooks, one for liquid formulations (Attachment 1) and the other for granular applications (Attachment 2). These exposure assessments are generally similar, but some of the computational details vary because of differences between granular and liquid formulations. In addition, there is a substantial difference in residue rates on contaminated vegetation, with much higher residues expected after foliar application of liquid formulations compared to those expected after soil application of granular formulations. For aquatic species, the concentrations in water are identical to those used in assessing exposures to both terrestrial wildlife and humans.

4.2.2. Terrestrial Animals

Most plausible exposures of terrestrial animals involve oral exposure, either from contaminated vegetation or contaminated water. The estimates of oral exposure are expressed in the same units as the available toxicity data. As in the human health risk assessment, these units are usually expressed as mg of agent per kg of body weight and abbreviated as mg/kg for terrestrial animals. For dermal exposures to terrestrial animals, the units of measure are expressed in mg of agent per cm^2 of surface area of the organism and abbreviated as mg/cm^2. In estimating dose, however, a distinction is made between the exposure dose and the absorbed dose. The *exposure dose* is the amount of material on the organism (i.e., the product of the residue level in mg/cm^2 and the amount of surface area exposed), which can be expressed either as mg/organism or mg/kg body weight. The *absorbed dose* is the proportion of the exposure dose that is actually taken in or absorbed by the animal.

In each workbook, the exposure assessments for terrestrial animals are summarized in Worksheet G01. The computational details for each exposure assessment presented in this section are provided as scenario specific worksheets (Worksheets F01 through F16b). Given the large number of species that could be exposed to pesticides and the varied diets in each of these species, a very large number of different exposure scenarios could be generated. For this generic risk assessment, an attempt is made to limit the number of exposure scenarios. The specific exposure scenarios developed in this section are designed as conservative screening scenarios, that may serve as guides for more detailed site-specific assessments by identifying the groups of organisms and routes of exposure that are of greatest concern.

Because of the relationship of body weight to surface area as well as to the consumption of food and water, small animals will generally receive a higher dose of imidacloprid, in terms of mg/kg body weight, than large animals for a given type of exposure. Consequently, most general exposure scenarios for mammals and birds are based on a small mammal or bird. For mammals, the body weight is taken as 20 grams, typical of mice, and exposure assessments are conducted for direct spray (F01 and F02a), consumption of contaminated fruit (F03, F04a, F04b), and consumption of contaminated water (F05, F06, F07). Grasses will generally have higher concentrations of herbicides than fruits and other types of vegetation (Fletcher et al. 1994). Because small mammals do not generally consume large amounts of grass, the scenario for the assessment of contaminated grass is based on a large mammal (Worksheets F10, F11a, and F11b). Other exposure scenarios for mammals involve the consumption of contaminated insects by a small mammal (Worksheet F14a) and the consumption of small mammals (contaminated via direct spray) by a large mammalian carnivore (Worksheet F16a). Exposure scenarios for birds involve the consumption of contaminated insects by a small bird (Worksheet F14b), the consumption of contaminated fish by a predatory bird (Worksheets F08 and F09), the consumption of small mammals (contaminated via direct spray) by a predatory mammal (Worksheet 16a) or a predatory bird (Worksheet 16b), and the consumption of contaminated grasses by a large bird (Worksheets F12, F13a, and F13b).

4.2.2.1. Direct Spray – In broadcast applications of any insecticide, wildlife species may be sprayed directly. This scenario is similar to the accidental exposure scenarios for the general

public discussed in Section 3.2.3.2. In a scenario involving exposure to direct spray, the amount absorbed depends on the application rate, the surface area of the organism, and the rate of absorption.

For this risk assessment, three groups of direct spray or broadcast exposure assessments are conducted (Worksheets F01, F02a, and F02b). For the granular formulations, a spray is not a meaningful concept. By analogy to residues on contaminated vegetation (Section 3.2.3.6), it is also likely that the granular formulations will not stick to mammals or other ecological receptors considered in this risk assessment. Because these differences cannot be quantified, however, exposures to granular formulations, like liquid formulations, are taken at the nominal application rate. As discussed further in Section 4.4, all risks are far below a level of concern and any over-estimate of exposure has no impact on the conclusions reached in the current risk assessment.

The first spray scenario, which is defined in Worksheet F01, involves a 20 g mammal that is sprayed directly over one half of the body surface as the chemical is being applied. This scenario assumes first-order dermal absorption. The second exposure scenario, detailed in Worksheet F02a, assumes complete absorption over day 1 of exposure. This very conservative assumption is likely to overestimate exposure and is included to encompass any increase in exposure due to grooming. The third exposure assessment is developed using a body weight of a honey bee, again assuming complete absorption of the compound. Direct spray scenarios are not given for large mammals; allometric relationships dictate that large mammals will be exposed to lesser amounts of a compound in any direct spray scenario than smaller mammals.

4.2.2.2. Indirect Contact – As in the human health risk assessment (see Section 3.2.3.3), the only approach for estimating the potential significance of indirect dermal contact is to assume a relationship between the application rate and dislodgeable foliar residue. Unlike the human health risk assessment in which transfer rates for humans are available, there are no transfer rates available for wildlife species. Wildlife, compared with humans, are likely to spend longer periods of time in contact with contaminated vegetation. It is reasonable to assume that for prolonged exposures an equilibrium may be reached between levels on the skin, rates of absorption, and levels on contaminated vegetation. No data regarding the kinetics of such a process are available, and in the absence of such data, no quantitative assessments are made for this scenario in the ecological risk assessment.

4.2.2.3. Ingestion of Contaminated Vegetation or Prey – In broadcast applications involving application directly to vegetation, the consumption of contaminated vegetation is an obvious concern and separate exposure scenarios are developed for acute and chronic exposure scenarios for a small mammal (Worksheets F04a and F04b), a large mammal (Worksheets F10, F11a, and F11b), and large birds (Worksheets F12, F13a, and F13b).

For imidacloprid, there are several aspects to these exposures assessments that are difficult to account for quantitatively. As discussed in the exposure assessment for human health (Section 3.2.3.6), there are likely to be substantial differences in residues on vegetation after broadcast applications of liquid as compared to granular applications. As in the human health risk assessment, these differences are reflected in the worksheets for broadcast applications of liquid

formulations (Attachment 1) and broadcast applications of granular formulations (Attachment 2).

As also discussed in the human health risk assessment (Section 3.2.3.6), applications of imidacloprid by soil injection and tree injection will lead to residues in the plant but amounts of these residues are difficult to quantify and will vary over time. Based on the study by Tattar et al. (1998), as discussed in Section 3.2.3.6, it seems likely that concentrations in fruit after direct spray of a liquid formulation will encompass plausible concentrations in treated trees after soil or tree injection applications of imidacloprid that are effective in adelgid control. Concentrations of imidacloprid in other plant species could be comparable but there is no data to support this supposition. This is discussed further in the risk characterization.

Similarly, the consumption of contaminated insects is modeled for a small bird (Worksheet 14a) and a small mammal (Worksheet 14b). As with residues on vegetation, data are available on residues of imidacloprid on insects. In the field study by Toll (1994), residues on terrestrial invertebrates were about 6.38 ppm after the application of imidacloprid at 0.4 lbs/acre to turf. Normalized for application rate, this corresponds to about 16 ppm per lb/acre. This is in the range of estimates from Fletcher et al. (1994) with default values of 7 ppm to 15 ppm at 1 lb/acre for large insects. Consistent with the approach taken in other Forest Service risk assessments, the empirical relationships recommended by Fletcher et al. (1994) are used as surrogates, as detailed in Worksheets F14a and F14b. Note that both of these worksheets model concentrations in small insects with residue rates of 45 ppm to 135 ppm per lb/acre. For liquid formulations (Attachment 1) this more conservative approach does impact the risk characterization for both small mammals and small birds (Section 4.4).

A similar set of scenarios is provided for the consumption of small mammals by either a predatory mammal (Worksheet 16a) or a predatory bird (Worksheet 16a). In addition to the consumption of contaminated vegetation, insects, and other terrestrial prey, imidacloprid may reach ambient water and fish. Thus, a separate exposure scenario is developed for the consumption of contaminated fish by a predatory bird in both acute (Worksheet F08) and chronic (Worksheet F09) exposures. Details of each scenario are given in the cited worksheets.

Multi-route exposures (e.g., the consumption of contaminated vegetation and contaminated water) are likely. Any number of combinations involving multiple routes of exposure could be developed. Such scenarios are not developed in the current risk assessment because the predominant route of plausible exposure is contaminated vegetation. Explicit considerations of multiple routes of exposure would have no impact on the characterization of risk.

4.2.2.4. Ingestion of Contaminated Water – Estimated concentrations of imidacloprid in water are identical to those used in the human health risk assessment (Table 3-3). The only major differences involve the weight of the animal and the amount of water consumed. These differences are detailed and documented in the worksheets that involve the consumption of contaminated water (F05, F06, F07). As in the human health risk assessment, different concentrations in water are estimated for broadcast applications of liquid formulations in clay or loam (Attachment 1), broadcast applications of granular formulations in clay or loam

(Attachment 2), soil injection in clay or loam (Attachment 3), and broadcast applications or soil injection in predominantly sandy soil.

4.2.3. Terrestrial Plants

Terrestrial plants, particularly hemlocks, will certainly be exposed to imidacloprid in any application that is effective in the control of adelgids. A large number of different exposure assessments could be made for terrestrial plants – i.e., direct spray, spray drift, runoff, wind erosion and the use of contaminated irrigation water. Such exposure assessments are typically conducted for herbicides. For imidacloprid, however, the development of such exposure assessments would serve no purpose. As discussed in Section 4.1.2.4 (Hazard Identification for Terrestrial Plants), there is no basis for asserting that imidacloprid will cause adverse effects in terrestrial plants. Thus, no formal exposure assessment is conducted for terrestrial plants.

4.2.4. Soil Organisms

A limited number of toxicity studies are available in which the toxicity of imidacloprid to soil organisms is expressed in units of soil concentration. The GLEAMS modeling discussed in Section 3.2.3.4 provides estimates of concentration in soil as well as estimates of off-site movement (runoff, sediment, and percolation). Based on the GLEAMS modeling, concentrations in clay, loam, and sand over a wide range of rainfall rates are summarized in Appendix 10 for broadcast applications of liquid formulations, Appendix 11 for broadcast applications of granular formulations, and Appendix 12 for soil injection. Table 4 in each of these appendices gives the estimate concentration of imidacloprid in the top 12 inches of the soil column at a normalized application rate of 1 lb/acre. Analogous to the approach taken with water contamination rates (Table 3-5), a summary of the modeled soil concentrations is presented in Table 4-5. Note that the concentrations in this table are given in units of mg imidacloprid/kg soil (ppm).

The peak soil concentrations show relatively little variability, in the range of 0.13 ppm to 0.26 ppm per lb/acre applied. All of these peak concentrations occur shortly after application. Soil injection and granular applications lead to higher soil residues than liquid applications because the modeling assumes that 50% of liquid applications are initially applied to vegetation. For that application rate of 0.4 lb imidacloprid/acre, the estimated peak soil concentrations are in the range of 0.05 ppm to 0.1 ppm. As discussed further in Section 4.4, the upper range of these concentrations approaches the NOAEC for sperm deformities in earthworms (Luo et al.1999). Longer term concentrations of imidacloprid in soil are substantially lower (in the range of 0.0006 to 0.07 ppm per lb/acre), and correspond to soil concentrations of about 0.00004 ppm to 0.03 ppm.

4.2.5. Aquatic Organisms

The assessment of the potential effects of imidacloprid on aquatic species is based on the concentrations of imidacloprid in water as developed in the human health risk assessment. These values are summarized in Table 3-6 and are discussed in Section 3.2.3.4.6. In each set of workbooks (Attachments 1 to 4), these concentrations are used in Worksheet G03 to characterize risk to aquatic species.

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Overview

The specific toxicity values used in this risk assessment are summarized in Table 4-6 and Table 4-7. Table 4-7 provides an overview of the toxicity values used for terrestrial invertebrates and the remaining toxicity values for other organisms are given in Table 4-6. The derivation of each of these values is discussed in the various subsections of this dose-response assessment. The available toxicity data support separate dose-response assessments in six classes of organisms: terrestrial mammals, birds, non-target terrestrial invertebrates, fish, aquatic invertebrates, and aquatic algae. Different units of exposure are used for different groups of organisms depending on how exposures are likely to occur and how the available toxicity data are expressed.

On the basis of both acute and chronic toxicity, the order of sensitivity to imidacloprid among terrestrial organisms is honey bees (most sensitive), followed by birds, and then mammals (least sensitive). The acute and chronic NOAEL values, respectively, are: 0.013 mg/kg and 0.010 mg/kg/day for honey bees; 3 mg/kg and 0.3 mg/kg/day for birds; and 0.14 mg/kg/day and 5.7 mg/kg for mammals.

Due to the number of studies in the open literature which attempt to assess the potential effects of imidacloprid on beneficial predatory arthropods other than honey bees, there are values for beneficial predators, which are presented in terms of application rate. These values are presented in Table 4-7 and are used to qualify and refine conclusions based on the the data for honey bees.

Both acute and chronic toxicity values for aquatic species indicate a large difference between fish and certain sensitive aquatic invertebrates. For fish, the acute NOAEC values are 25 mg/L and 50 mg/L for sensitive and tolerant species, respectively. For invertebrates, the corresponding acute NOAEC values are 0.00035 mg/L and 145 mg/L. For fish, a chronic NOAEC of 9.8 mg/L is available from a chronic life-stage study. Chronic NOAEC values of 0.000163 mg/L and 1.8 mg/L are used for sensitive and tolerant aquatic invertebrates, respectively. Toxicity values of 6.69 mg/L (sensitive) and 119 mg/L (tolerant) are used for aquatic algae. Because of the short life-cycle of individual algal cells, the relatively short-term bioassays in algae (i.e., 96 to 120 hours) are applied to both acute and longer-term concentrations for the characterization of risk.

On the basis of acute toxicity, amphibians are less sensitive than mammals, fish, and sensitive aquatic invertebrates. Acute NOEC values of 30 mg/L and 101.2 mg/L are used in this assessment for sensitive and tolerant amphibian species, respectively. For longer-term exposures, NOEC values of 17.5 mg/L and 88 mg/L are used for sensitive and tolerant species, respectively.

The risks associated with metabolites of imidacloprid are not addressed directly or quantitatively in this assessment. In mammals, fish, and aquatic invertebrates, no metabolite tested was shown to cause toxicity at lower concentrations than the parent imidacloprid compound. In insects the olefin, 5-hydroxy and 4,5-di-hydroxy-metabolites were shown to be active in causing toxicity at

or below the concentrations at which imidacloprid causes adverse effects. Although it has been hypothesized that these metabolites might be responsible for the delayed mortality observed in many acute studies with insects following exposure to imidacloprid, it is assumed that any benchmark values protective of the adverse effects of imidacloprid will also be protective of it's metabolites. Therefore, toxicity values for individual imidacloprid residues are not derived in this assessment.

4.3.2. Toxicity to Terrestrial Organisms

4.3.2.1. Mammals – As summarized in the dose-response assessment for the human health risk assessment (see Section 3.3.3.), the most sensitive chronic effects in experimental mammals are reduction in body weight and changes in thyroid tissue. The chronic NOAEL for these endpoints in experimental mammals is 5.7 mg/kg/day (U.S. EPA/OPP 2003) and is based on a studies in rats (Eiben and Kaliner 1991; Eiben 1991). In the first study, male and female Wistar rats were fed dietary concentrations of 0, 100, 300 and 900 ppm technical grade imidacloprid for 24 months (Appendix 2). These dietary concentrations correspond to mean measured doses of 0, 5.7, 16.9 and 51.3 mg/kg body weight per day for males and 0, 7.6, 24.9 and 73.0 mg/kg body weight per day for females. Treatment-related increases in the incidence of mineralization of the colloid of the thyroid follicles was observed in males at 300 and 900 ppm, and in females at 900 ppm. Treatment-related reductions in body weight gain were observed at 900 ppm in both sexes. The second study by Eiben (1991) confirmed the effects on body weight and the thyroid (Appendix 2). Groups of male and female Wistar rats were fed 0 or 1800 ppm technical grade imidacloprid in the diet for 24 months. This corresponded to doses of 0 and 102.6 mg/kg/day for males, and 0 and 143.7 mg/kg/day for females. An increased incidence of thyroid changes (mineralization of colloid; fewer colloid aggregation sites; parafollicular hyperplasia sites with minimal intensity) and reduction in body weight gain were observed in both sexes.
Thus, for this risk assessment, 5.7 mg/kg/day is taken as the chronic NOAEL for general toxic effects.

Consistent with the approach taken in the human health risk assessment (Section 3.3.4), acute (1-day) exposures will be based on the acute LOAEL of 42 mg/kg from the acute neurotoxicity screening studies on rats (Sheets 1994a,b). Dividing the LOAEL by an uncertainty factor of 3 (U.S.EPA/OPP 2003) yields a NOAEL of 14 mg/kg. Thus, 14 mg/kg is used as the acute NOAEL for mammals.

4.3.2.2. Birds – As detailed in Appendix 3 and summarized further in Table 4-1, adverse reproductive effects were observed in mallard ducks and bobwhite quail, with bobwhite quail being the more sensitive species. In mallards, a significant reduction in mean number of eggs laid per hen was observed at a dietary concentration of 234 ppm, but not at 125 ppm (Toll 1991c). In another one-generation study with mallard ducks, a statistically significant reduction in eggshell thickness and strength was observed at 250 ppm, but not at 128 ppm (Stafford 1992). In a one-generation study with bobwhite quail, a significant reduction in hatchling body weight was observed in comparison with controls at all dietary concentrations, yielding a LOAEC of 36 ppm imidacloprid in the diet (Toll 1991b). Using experimental data from Toll (1991b) it is possible to convert the dietary concentration of 36 ppm to a dose. On average, birds in the 36 ppm dietary exposure group ingested 18 grams of food per day, and female birds had an average

weight of 288 grams. Multiplying 36 mg imidacloprid/kg diet by 0.018 kg diet/day, and dividing by 0.288 kg/bird, yields a LOAEL of 2.25 mg/kg/day.

On the basis of acute exposure, bobwhite quail and mallard duck are among the least sensitive species tested. As shown in Table 4-1, canaries, house sparrows and Japanese quail all had acute NOAEL values (10 mg/kg, 3 mg/kg and 3.1 mg/kg, respectively) approximately three to ten times lower than the acute NOAEL for bobwhite quail (25 mg/kg).

This assessment uses the acute NOAEL of 3 mg/kg from the study on house sparrows (Stafford 1991) to assess the potential effects of short-term (1-day) exposure to imidacloprid on birds. This assessment uses a chronic NOAEL of 0.3 mg/kg/day to assess potential impacts of long-term exposure on birds. The chronic NOAEL is based on consideration of the LOAEL derived from the one-generation study on bobwhite quail (Toll 1991b) and the acute NOAEL of 3 mg/kg for house sparrows (Stafford 1991), as follows. Given that: 1) no longer-term reproduction studies were conducted for songbirds, and 2) songbirds appear to be more sensitive than bobwhite quail on an acute basis, it is appropriate to take into consideration the acute toxicity values in determining the chronic NOAEL for this assessment. Dividing the acute NOAEL of 3 mg/kg for house sparrows by an uncertainty factor of 10 (extrapolation from acute to chronic exposure) would yield a chronic NOAEL of 0.3 mg/kg/day. Dividing the chronic bobwhite LOAEL of 2.25 mg/kg/day by an uncertainty factor of 10 would yield a NOAEL of 0.225 mg/kg/day. In the Toll (1991) study with bobwhite quail, the investigators did not consider the significant reduction in hatchling body weights to be biologically meaningful, because 14-day survivor weights in higher dosed birds were equal to or higher than that of controls. However, the effect at hatching was real, if possibly transient. Thus, one could make the argument that dividing the LOAEL by less than a full uncertainty factor of ten is appropriate in this case, similar to the approach taken by U.S. EPA in deriving the acute RfD (Section 3.3.2). Dividing the LOAEL of 2.25 by an uncertainty factor of 6.75 would yield a NOAEL of 0.3. Given the transient nature of the observed effect in quail and taking into consideration the chronic NOAEL which can be extrapolated from the acute NOAEL for the most sensitive species on an acute basis, it seems appropriate to choose 0.3 mg/kg/day as the chronic NOAEL for birds. Based on these considerations, this assessment selects a chronic NOAEL of 0.3 mg/kg/day as the basis for estimating longer-term exposure of birds to imidacloprid.

4.3.2.3. Terrestrial Invertebrates – As discussed in Section 4.1.2.3, imidacloprid is an insecticide which works through activation of nicotinic acetylcholinesterase receptors, ultimately causing paralysis and death. Insects, beneficial or otherwise, are thus the most sensitive organisms to imidacloprid exposure. A large number of diverse studies have been conducted on the effects of imidacloprid on insects, due to the fact that imidacloprid was one of the first neo-nicotinoid insecticides developed. Details of the available studies are presented in Appendix 4 and are discussed in Section 4.1.2.3. The discussion below focuses on those studies and endpoints that are used quantitatively in the dose-response assessment for terrestrial invertebrates and these studies are summarized in Table 4-7, which covers the following groups: bees, beneficial predatory insects, ants, and earthworms. Table 4-7 also includes a toxicity value for fungi that is discussed further in Section 4.3.2.4.

Honey bees appear to be very sensitive to imidacloprid. The NOAEL of 1.2 ng/bee (mortality) is used as the basis for the assessment of risk in short-term exposures. Using a body weight of 0.000093 kg for the honey bee (USDA/APHIS 1993), the NOAEL of 1.2 ng/bee (Schmuck et al. 2001) is multiplied by 0.000001 mg/ng and divided by 0.000093 kg to arrive at a dose-based NOAEL of 0.013 mg/kg. This value is supported by a chronic dietary NOAEC of 24 ug/kg (Decourtye et al. 2003) which can be converted to an equivalent dose (NOAEL) of 0.010 mg/kg/day (Table 4-7), and a field study in bumble bees by Gels et al. (2002), discussed further below.

Other studies on honey bees conducted by Decourtye et al (2003) and Guez et al (2001) suggest that laboratory-conditioned sub-lethal effects on learning associated with feeding behavior (proboscis extension reflex habituation, and olfactory learning, for example) may occur at this dose or lower doses, but the relevance of these studies to actual results in the field remains in question. In fact, a study conducted by Decourtye et al. (2004) with honey bees in outdoor flight cages demonstrates no significant difference in foraging (measured by sucrose consumption) between controls and imidacloprid-exposed bees at imidacloprid concentrations as high as 24 ug/kg diet (equivalent to approximately 1 ng/bee/day or 0.011 mg/kg/day). A chronic field study conducted by Gels et al.(2002) with bumble bees also failed to detect any adverse impacts on bumble bees (foraging, colony vitality) exposed to imidacloprid applied via spray or granular formulations equivalent to 0.336 kg a.i./ha, so long as the application was followed with appropriate irrigation or rainfall.

The available field- and simulated field- studies suggest that application of imidacloprid at a rate equivalent to that those proposed by the Forest Service (0.5 lb a.i./acre) could result in reduced survival among honey bees, sensitive parasitic wasps, such as *Diadegma insulare* (Hill and Foster 200), or predatory bugs such as *Orius laevigatus* (Delbecke et al. 1997). On the other hand, predatory ants do not seem to be affected by imidacloprid applied at label recommended rates (Kunkel et al. 1999; Zenger and Gibb 2001).

As discussed in section 4.1.2.3, Hancock et al. (1992) sprayed alfalfa with imidacloprid at rates of 0.045, 0.167 and 0.5 lb a.i./acre, and determined the residual time needed to reduce chemical activity such that honey bee mortality was less than 25% (RT_{25}). The estimated RT_{25} values are: <2 hours, < 8 hours, and 8 hours, for application rates of 0.045, 0.167, and 0.5 lb a.i./acre, respectively. The RT_{25} of < 2 hours for 0.045 lb a.i./acre indicates that imidacloprid may be applied at this rate of with minimal hazard to bees during early morning, or late in the evening when bees are not actively foraging. The RT_{25} < 8 hours associated with 0.167 lb a.i./acre indicates that imidacloprid may be applied at this rate with minimal hazard to bees late in the evening when bees are not actively foraging. The RT_{25} = 8 hours associated with 0.5 lb a.i./acre indicates that imidacloprid may be applied at this rate with moderate hazard to bees late in the evening when bees are not actively foraging.

Toxicity to soil invertebrates will be based on an assay in earthworms (Luo et al. 1999, discussed in Section 4.1.2.3) in which no effects on sperm deformity were noted over a 14-day exposure period at soil concentrations of up to 0.1 ppm (0.1 mg/kg soil) but effects were seen at concentrations of 0.5 ppm and higher.

4.3.2.4. Terrestrial Plants – As discussed in Sections 4.1.2.4, there is no reason to assume that imidacloprid will cause adverse effects in terrestrial plants. No standard toxicity studies have been encountered that could be used to quantify risk in either terrestrial plants. The studies that are available (e.g., Webb et al. 2003; Westwood et al. 1988) indicate that imidacloprid is not phytotoxic under conditions of normal use.

4.3.2.5. Terrestrial Microorganisms – As noted in Section 4.1.2.5, very few quantitative bioassays on the toxicity of imidacloprid have been encountered in the literature. Imidacloprid applied to sandy soil at a rate of 10 mg a.i/kg soil was shown to inhibit fungal growth but not bacterial growth with respect to untreated control soil, after 2-weeks of incubation in laboratory conditions (Tu 1995).

4.3.3. Aquatic Organisms

4.3.3.1. Fish – The acute bioassays on fish summarized in Appendix 7 provide estimates of exposures which might be associated acute effects in two species of freshwater and one species of saltwater fish. The most sensitive species is the bluegill sunfish with a 96-hour NOAEC of 25 mg/L (Bowman and Bucksath 1990a). Rainbow trout appear to be somewhat less sensitive, with a 96-hour NOAEC of 50 mg/L (Grau 1988a). For this risk assessment, the NOAEC values of 25 mg/L and 50 mg/L are used to assess the consequences of short-term exposures for sensitive and tolerant species.

The assessment of the effects of imidacloprid that might be associated with chronic exposure to contaminated ambient water from the normal use and application of this product is based on the 98-day early life-stage study on rainbow trout (Cohle and Bucksath 1991). A statistically significant reduction in body length was observed in fry at 36 and 60-days post-hatch, resulting in a LOAEC of 19 mg/L for the study. There were no statistically significant effects on egg viability, hatch, survival or behavioral variables. The NOAEC for the study is 9.8 mg/L. The NOAEC of 9.8 mg/L is used in this assessment to evaluate the potential effects of long-term imidacloprid exposure on both sensitive and tolerant species of fish.

4.3.3.2. Amphibians – As discussed in Section 4.1.3.2 and detailed in Appendix 7, studies on amphibians address acute mortality in two species of frogs (Feng et al. 2004), and hatchling success and development in two species of frogs, a salamander, and a toad (Julian and Howard 1999).

For evaluating short-term exposure, this assessment uses the 96-hour NOAEC of 30 mg/L (frog, *Rana linocharis*) for the sensitive species, and the 96-hour NOAEC of 101.2 mg/L (frog, *Rana hallowell*) for the tolerant species (Feng et al. 2004).

The study by Julian and Howard 1999 provides information from which chronic NOAEC values for amphibians are derived. No effects on hatchling success or significant differences between imidacloprid-exposed tadpoles and controls with regard to individual or total deformities were observed at any concentration tested. Concentrations used in the study were determined on the basis of LC_{50} values previously reported for ranids, and thus, should have been high enough to cause adverse effects if they were likely to occur in response to exposure. Only one of the

species tested, the chorus frog, *Pseudacris triseriata* , had a high percentage of total deformities (mean value of 24%, with a range of approximately 23 to 25 %) which approached but did not achieve a statistically significant difference from control values (11.2%, with an approximate range of 2.5 to 15%) at the highest imidacloprid concentration tested (88-100 mg/L). However, it is possible that the high variability in the control percentage prevented statistical significance, and that the next lowest concentration (17.5-20 mg/L) is actually the NOAEC for *Pseudacris*. On this basis, 17.5 mg/L is selected as the chronic NOAEC for evaluating sensitive amphibian species. On the basis of the lowest percentages of deformities with respect to controls, the toad, *Bufo americanus*, was the least sensitive, with a NOAEC of 88-110 mg/L. The chronic NOAEC of 88 mg/L is therefore used to evaluate tolerant amphibian species.

4.3.3.3. Aquatic Invertebrates – As discussed in Section 4.1.3.3, standard laboratory studies on freshwater and saltwater species, as well as a microcosm study, have been conducted with technical grade imidacloprid. An overview of the key toxicity values from these studies is given in Table 4-4 and additional details are presented in Appendix 6.

On the basis of both acute and chronic toxicity, crustaceans and aquatic insects are more sensitive to imidacloprid than fish. Amphipod crustaceans such as *Hyalella azteca*, the saltwater Mysid, *Mysidopsis bahia*, and the insect midge, *Chironomus tentans*, are the most sensitive species. In freshwater, the water flea, *Daphnia magna*, was the least sensitive species, while in saltwater, the eastern oyster was least sensitive. Acute toxicity values range from a 96-hour NOAEC of 0.00035 mg/L for *Hyalella azteca* (England and Bucksath 1991), to a 96-hour NOAEC of 145 mg/L for eastern oyster (Wheat and Ward 1991). On the basis of these studies, NOAEC values of 0.00035 mg/L and 145 mg/L are chosen to evaluate acute exposure of sensitive and tolerant aquatic invertebrate species, respectively.

On the basis of longer-term studies designed to assess reproduction, growth and survival, *Mysidopsis bahia* was the most sensitive species, with a NOAEC value of 0.000163 mg a.i. imidacloprid/L for growth and reproductive success (Ward 1991), and *Daphnia magna* was the most tolerant species with a 21-day NOAEC for immobility of 1.8 mg/L (Young and Blake 1990).

A 19-week microcosm study conducted as part of EPA's pesticide registration requirements for imidacloprid confirms the sensitivity of amphipods and midges observed in laboratory studies (Moring et al. 1992). Technical grade imidacloprid was applied to the surface of tanks containing a variety of phytoplankton, zooplankton, and macro-invertebrates at two week intervals, for a total of 4 applications. Concentrations ranging from 0 to 0.180 mg a.i./L were employed. Amphipods were determined to be the most sensitive species, with statistically significant impacts on abundance at some sampling intervals at the lowest concentration tested, yielding a LOAEC of 0.002 mg a.i./L. Statistically significant decreases in populations of total macro-invertebrates as well as individual macro-invertebrate taxa (mayfly, midge, caddisfly, beetle and amphipod) were most frequently observed (at different sampling endpoints) at imidacloprid concentrations ranging from 0.02 to 0.180 mg a.i./L. On the basis of these findings, the study authors recommended 0.006 mg a.i./L as a regulatory NOAEC for imidacloprid in aquatic systems. However, the results of laboratory studies (Gagliano 1991;

Ward 1991; England and Bucksath 1991), as well as the results for amphipods at some sampling intervals in this study, suggest that the NOAEC for growth and survival of sensitive macro-invertebrate species is on the order of 0.000163 mg a.i./L. With these considerations in mind, the NOAEC values of 0.000163 (*Mysidopsis bahia* reproductive success, Ward 1991) and 1.8 mg/L (*Daphnia magna* immobility, Young and Blake 1990) are used in this assessment to evaluate potential effects of longer-term exposure to imidacloprid on sensitive and tolerant aquatic invertebrates, respectively.

4.3.3.4. Aquatic Plants – As discussed in Section 4.1.3.4, several standard studies are available on the toxicity of imidacloprid to aquatic plants. As would be expected for a neurotoxic insecticide, aquatic plants are much less sensitive to imidacloprid than fish or aquatic invertebrates. While Moring et al. (1992) report sporadic decreases in cyanophyte populations over the course of a microcosm study at concentrations of 0.020 mg/L and higher, the controlled laboratory bioassay by Bowers et al. (1996b) demonstrates an NOEC for a blue-green algae, *Anabaena flos-aquae*, of 24.9 mg a.i./L. Given the transient nature of the observations in the mesocosm study, the decreases in cyanophyte populations in the study by Moring et al. (1992) appear to be incidental.

For this risk assessment, NOEC for sensitive species will be set at of 6.69 mg/L based on the study with *Navicula pelliculosa* by Hall (1996). The NOEC for tolerant species will be set at 119 mg/L based on the study in *Selenastrum capricornutum* by Gagliano and Bowers (1991).

4.4. RISK CHARACTERIZATION

4.4.1. Overview

As with the human health risk assessment, the risk characterization for imidacloprid depends on the application method. The Forest Service will typically restrict applications of imidacloprid to either tree injection or soil injection in clay or loam soils. Neither of these application methods are likely to cause adverse effects in nontarget species. Broadcast applications of imidacloprid may be considered by some groups working in cooperation with the Forest Service. Broadcast applications will result in higher exposures to nontarget species and some adverse effects are plausible.

Tree injection of imidacloprid is highly specific and will not result in substantial exposures to nontarget species. The only plausible exception would be beneficial insects that prey on adelgids or other similar pest insects. In such cases, effects on these beneficial insects might occur. Field studies have demonstrated adverse effects on some beneficial insects but these effects tend to be transient.

Soil injection of imidacloprid is also a relatively specific application method and exposures to most nontarget species will be far below a level of concern. An obvious exception, however, involves soil dwelling organisms such as earthworms, soil arthropods, and soil microorganisms. After soil injection, concentrations of imidacloprid will be in the range of soil concentrations that have been shown to cause sperm deformity in earthworms. In addition, field studies have demonstrated decreases in earthworm populations after applications of imidacloprid comparable to rates used in Forest Service programs. These effects, however, appear to be transient. There is little indication that imidacloprid is likely to cause adverse effects on soil microorganisms. Concentrations of imidacloprid could approach or somewhat exceed those associated with decreases in populations of soil fungi (but not soil bacteria). Again, these effects will be transient and concentrations of imidacloprid in soil will decrease to levels below those that might be associated with effects in fungi.

Broadcast applications of granular or liquid formulations will result in much greater exposures to a wider variety of nontarget species. The greatest difference between granular and liquid formulations will involve residues on vegetation and insects. Liquid formulations are likely to result in substantially greater residues than granular formulations. The broadcast application of liquid formulations lead to acute hazard quotients that exceed a level of concern for a large mammal consuming vegetation (HQ=1.4), a small mammal consuming insects (acute HQ=2), and large birds consuming grass (HQ=10). For sensitive bird species, the broadcast application of liquid formulations of imidacloprid could be associated signs of frank toxicity and possibly with substantial mortality after acute exposures.

The longer-term consumption of contaminated vegetation by a large bird also exceeds the level of concern (HQ=1.7). The effects associated with longer-term exposures are regarded as undesirable but the effects, such as weight loss, are not likely to be severe. There is no indication that frank adverse effects such as obvious debilitation or mortality would be observed.

Imidacloprid is not very toxic to fish, amphibians, and even some aquatic invertebrates. No effects on any aquatic species are likely after either tree injection or soil injection applications to predominantly clay or loam soils. In addition, worst-case estimates of peak or longer-term exposures from broadcast applications suggest that adverse effects are not likely to occur in aquatic vertebrates. Differences between sensitive and tolerant aquatic invertebrate species are substantial, spanning a factor of over 400,000 for acute NOEC values and over 11,000 for longer-term NOEC values. Depending on the application method and soil type, hazard quotients for sensitive aquatic invertebrates could range from about 2 to over 80.

As in the human health risk assessment, the ecological risk assessment uses a scenario for an accidental spill that involves the contamination of a small body of water with 0.4 lb to 40 lbs of imidacloprid. Over this range, the hazard quotients for sensitive aquatic invertebrates are extraordinarily high, ranging from about 500 to over 50,000. While the likelihood and plausibility of such spills may be remote, these hazard quotients clearly suggest that the greatest risk in the event of an accidental spill will be to aquatic invertebrates. As with fish and amphibians, tolerant aquatic invertebrates are not at risk in the event of an extreme spill.

4.4.2. Terrestrial Organisms

4.4.2.1. Mammals – The risk characterization for mammals as well as other terrestrial organisms is summarized in Worksheet G02 of the workbooks that accompany this risk assessment. As in the human health risk assessment, different versions of this worksheet are contained in four workbooks: broadcast liquid applications (Attachment 1), broadcast granular applications (Attachment 2), soil injection applications (Attachment 3), and applications to predominantly sandy soil (Attachment 4). For mammals as well as other groups of organisms considered below, all hazard quotients are based on the maximum single application rate of 0.4 lb/acre.

For soil injection, an application method that will be used in Forest Service programs, none of the hazard quotients exceed a level of concern. As in the human health risk assessment (Section 3), no explicit exposure assessments are made for tree injection, another application method that may be commonly used in Forest Service programs. Tree injection applications are likely to result in lower exposures than those associated with soil injection. Given the very low hazard quotients associated with soil injection, no plausible risks to mammals are apparent for tree injection.

A possible exception to this assessment involves exposures associated with animals that may browse on treated hemlock. Several species of mammal consume various portions of hemlock trees. Because imidacloprid is translocated systemically throughout the tree, this would result in exposures to imidacloprid, regardless of the method of application. Among the preferred foods of the porcupine (*Erethizon dorsatum*) are the inner bark, small twigs and buds of the eastern hemlock, consumed throughout the year. In winter the hemlock twigs are a preferred food of whitetail deer (*Odocoileus virginianus*). Snowshoe hare (*Lepus americanus*) will occasionally consume the bark of younger trees.

As discussed in Section 4.2.2.3, both soil injection and tree injection applications of imidacloprid that are effective in the control of adelgids will result in uptake of imidacloprid by hemlock. These exposures cannot be quantified well because of uncertainties in the amount of treated vegetation that a mammal or bird might consume. This risk assessment uses exposure assessments for grazers of directly treated vegetation. As noted in Section 4.2.2.3, browsers on treated hemlock may be exposed to concentrations of imidacloprid that are comparable to those on fruit after a direct spray. However, no reliable estimates are available on the amount of treated hemlock foliage that might be consumed. While somewhat speculative, it seems plausible the that risks to mammals associated with the consumption of contaminated fruit after liquid applications would encompass and may substantially exceed those associated with browsing on treated hemlock.

Broadcast applications of imidacloprid are likely to result in much higher exposures for mammals than either soil injection or tree injection. As detailed in Section 4.2, three general types of exposure scenarios are considered: direct spray, contaminated water, and contaminated food (vegetation or insects). None of the exposure scenarios associated with direct spray or contaminated water reach a level of concern (i.e., HQ=1). Of these scenarios, the highest hazard quotient is 0.7 (the direct spray of a small mammal assuming 100% absorption).

Two acute exposure scenarios for broadcast applications of a liquid formulation exceed a level of concern for mammals: the upper bound for the consumption of contaminated vegetation by a large mammal (HQ=1.4) and the upper bound for the consumption of contaminated insects by a small mammal (HQ=2). Both of these exposures are based on standard estimates of residues from Fletcher et al. (1994). As noted in Section 3.2.3.6, these residue rates are consistent with monitored residues of imidacloprid on vegetation.

The hazard quotients of 1.4 and 2 are associated with doses of 19.4 mg/kg and 27.8 mg/kg (Worksheet G01 of Attachment 1). These doses are above the estimated NOAEL of 14 mg/kg for neurotoxicity but below the corresponding LOAEL of 42 mg/kg. Thus, the consequences of these exposures cannot be clearly characterized.

4.4.2.2. Birds – The risk characterization for birds is summarized in Worksheet G02 of the workbooks that accompany this risk assessment. For the application methods that are likely to be used most often in Forest Service programs – i.e., tree injection and soil injection – plausible exposures and risks are likely to be negligible. As in the human health risk assessment, consideration of tree injection applications includes an accidental exposure scenario in which 0.4 lb to 40 lbs of imidacloprid are spilled into a small pond. The upper range of this exposure scenario yields a hazard quotient of 3.

Broadcast applications of imidacloprid will result in higher levels of exposure and risks. Nonetheless, broadcast applications of granular applications do no result in hazard quotients that exceed a level of concern except for the accidental spill of imidacloprid into a small pond. This accidental exposure scenario is identical to that used for soil injection – i.e., a spill of 0.4 lb to 40 lbs – and the upper range of the hazard quotient is also 3.

Liquid broadcast applications will result in higher levels of imidacloprid on contaminated vegetation than those associated with granular broadcast applications. For liquid broadcast applications, the the hazard quotients associated with the acute consumption of contaminated vegetation (grass) by a large bird ranges from 4 to 10. These hazard quotients are associated with doses of about 11 mg/kg and 30 mg/kg. As discussed in Section 4.3.2.2, there appears to be considerable variability in sensitivity among different types of birds – i.e., NOAELs of 3 to 25 mg/kg. For some sensitive species such as the canary (Grau 1994b, Appendix 3), doses of 10 to 30 mg/kg encompass a range of responses from signs of neurotoxicity to lethality – i.e., the LD_{50} for imidacloprid in the canary is in the range of 25-50 mg/kg bw. Thus, for sensitive bird species, the broadcast application of liquid formulations of imidacloprid could be associated signs of frank toxicity, and possibly with substantial mortality after acute exposures.

As also noted in Worksheets G02 of Attachment 1, longer-term exposures for a large bird consuming contaminated grasses exclusively at the application site lead to hazard quotients ranging from 0.6 to 16 with a central estimate of 1.7. These scenarios are associated with doses of about 0.2 mg/kg/day to 5 mg/kg/day with a central estimate of 0.5 mg/kg/day (Worksheet G01, Attachment 1). As discussed in Section 4.3.2.2, the longer-term toxicity value for birds is based on the 20-week reproduction study in bobwhite quail by Toll (1991b) in which the birds were fed concentrations of imidacloprid in the diet of 0, 30, 60, 120 and 240 ppm. Based on decrease hatchling weight at 30 ppm, this risk assessment uses 30 ppm as an LOAEL. Based on reported body weight and food consumption, this corresponds to a dose of about 2.25 mg/kg/day. Thus, at the upper range of exposure in this scenario (i.e., 5 mg/kg/day), a decrease in hatchling weight appears plausible. As noted in Appendix 6, however, 14-day survivor weights were normal at 30 and 60 ppm, and increased at dietary concentrations of 120 and 240 ppm. Thus, the toxicologic significance of the decreased hatchling weight is unclear.

The risks to birds associated with the consumption of contaminated vegetation, particularly grasses, should be modified with an assessment of the plausibility of such exposures. Hemlock characteristically occur in the late successional stages of forest ecosystems and grasslands are generally not part of such ecosystems. Consequently, the plausibility of the large bird model consuming treated grasses is very limited in the case of using imidacloprid on hemlocks.

4.4.2.3. Terrestrial Invertebrates – Imidacloprid is an effective insecticide that is designed to kill pest insects. Thus, in cases of exposures that are effective in killing target insects, adverse effects on non-target insects may be expected. This is illustrated in Worksheet G02 for the honey bee. In broadcast applications at a rate of 0.4 lb/acre, the hazard quotient for the honey after a direct spray is close to 5000. In other words, if a honey bee is directly sprayed with imidacloprid, it will probably die. This risk characterization, however, applies only to broadcast applications. For the applications that are anticipated in Forest Service programs – i.e., tree injection and soil injection – honey bees (an other insects) will not be sprayed and thus the risks associated with direct spray are not relevant to these application methods.

The severe risk characterization for flying insects after broadcast applications can be impacted by the timing of the application. As discussed in Sections 4.1.2.3 and 4.3.2.3, broadcast applications of up to 0.5 lb/acre (somewhat higher than the rate 0.4 lb/acre considered in this risk

assessment) could be applied without substantial hazard to honey bees if the application is made late in the evening when bees are not actively foraging (Hancock et al. 1992).

Risks to other insects, such as beneficial predatory arthropods, appear to be minimal based on the field studies by Kunkel et al. (1999) and Zenger and Gibb (2001). In cases where damage to beneficial insects has been noted, the damage appears to be transient – i.e., after a short period of time the population may rebound to exceed control values in terms of abundance and fecundity (see Section 4.1.2.3 and Appendix 4 for details). For example, Walthall and Stark (1997a,b) demonstrated that while pea aphid populations raised on imidacloprid-sprayed pea plants had increased mortality with respect to controls (72-hour LC_{50} values for neonates and adults were 0.225 mg/L and 0.468 mg/L, respectively) the surviving aphids had an increased reproduction rate which allowed them to maintain and/or exceed the population with respect to unexposed controls. Similar results (transient reduction followed by increased abundance) were demonstrated for Vedalia beetles (Grafton-Cardwell and Gu 2003) and hister beetles (Kunkel et al. 1999). Walthall and Stark (1997a) concluded that the observed effects of imidacloprid were due exclusively to the effects of mortality and the ability of the survivors to compensate through a higher rate of reproduction. The authors hypothesize that a higher rate of reproduction is possible among the survivors because of more abundant resources, and presumably, less competition for them due the initial decline in population with respect to controls. Walthall and Stark (1997a) do not attribute the recovery to a genetic component or genetic mutation involving resistance.

Because imidacloprid is most often applied to soil, organisms that live in soil may be subject to relatively high exposures. The most relevant study for quantifying effects in soil organisms is the NOAEC for sperm deformity in earthworms of 0.1 mg/kg dry soil. Adverse effects were noted at concentrations of 0.5 mg/kg and higher (Luo et al. 1999). As noted in Table 4 of Appendices 10 to 12, peak soil concentrations in the top 12-inches of treated soil will be in the range of 0.1 to 0.2 ppm, at or somewhat above the NOAEC of 0.1 ppm. Much lower concentrations of imidacloprid will be found deeper in the soil layer. Longer-term concentrations of imidacloprid in the top 12 inches of soil will be below the NOAEC of 0.1 ppm – i.e., in the range of 0.01 to 0.07 ppm. Thus, any effects on earthworms are likely to be transient. This is consistent with the field study by Kunkel et al. (1999) which noted only transient effects on earthworm populations when imidacloprid was applied at a rate of 0.45 kg/ha (0.4 lb/acre).

4.4.2.4. Terrestrial Plants – No quantitative risk assessment to terrestrial plants is made for imidacloprid. As discussed in Section 4.1.2.4, imidacloprid is not phytotoxic under conditions of normal use. In addition, imidacloprid has been extensively tested in both the laboratory and field studies for efficacy in the protection of terrestrial plants from insect pests. If imidacloprid were toxic to plants at applications in the range of those used to control the pest species, it is likely that reports of such phytotoxicity would be noted. No such reports have been encountered.

4.4.2.3. Soil Microorganisms – There is no indication that imidacloprid is likely to cause adverse effects on soil microorganisms. As discussed above (Section 4.4.2.3), peak

concentrations of imidacloprid in the top 12 inches of soil are likely to range from about 0.1 to 0.2 ppm. This is substantially below the 10 ppm NOAEC for soil bacteria and LOAEC for soil fungi noted in the study by Tu (1995). In addition, imidacloprid has been extensively tested for efficacy in protecting plants against insect pests. If imidacloprid caused significant adverse effects on soil microorganisms that are important for plant growth, this would probably have been noted in the literature. No such reports have been encountered.

4.4.3. Aquatic Organisms

4.4.3.1. Fish – As discussed in Section 4.1.3.1, imidacloprid is classified as practically nontoxic to fish. NOAEC values of 25 mg/L and 50 mg/L are used to assess the consequences of short-term exposures for sensitive and tolerant species, respectively. For longer-term exposures, an NOEC value of 9.8 mg/L based on a standard early life-stage study is used to characterize risks. All of these concentrations are substantially below plausible levels of exposure to imidacloprid by any application method. For soil injections (Attachment 3, Worksheet G03), the upper range of peak and longer-term hazard quotients are 0.00000002 and 0.000000001, below the level of concern (HQ=1) by factors of 50 million and 1 billion respectively. While broadcast applications lead to higher concentrations (Worksheet G03 of Attachments 1 and 2), the hazard quotients for peak and longer-term exposures are below the level of concern by factors of 1000 or more.

As noted above, an extremely conservative accidental exposure scenario for water contamination is used in this risk assessment – i.e., the spill of up to 40 lbs of imidacloprid into a small pond. For fish, even this very extreme scenario does not trigger a level of concern – i.e., the highest hazard quotient is 0.7.

4.4.3.2. Amphibians – Based on the data that are available, amphibians appear to be somewhat less sensitive to imidacloprid than fish. Consequently, as with fish, all hazard quotients are far below the level of concern.

4.4.3.3. Aquatic Invertebrates – Some aquatic invertebrates are much more sensitive to imidacloprid than fish or amphibians. As noted in Section 4.3.3.3, the differences between sensitive and tolerant invertebrate species are substantial, spanning a factor of over 400,000 for acute NOEC values and over 11,000 for longer-term NOEC values. Tolerant aquatic invertebrates appear to be equally or somewhat less sensitive to imidacloprid than fish or amphibians. Because of these differences, the risk characterization for aquatic invertebrates is highly dependent on both the sensitivity of the invertebrate and the the application method. This distinction is important because the Forest Service will typically use imidacloprid in soil injection applications to predominantly clay or loam soils and risks to aquatic invertebrates from such applications are negligible.

For soil injection in predominantly clay or loam soils (i.e., the applications that will be most commonly conducted in Forest Service programs), the hazard quotients for plausible (non-accidental) peak and longer-term exposures in sensitive species are 0.001 and 0.00007, respectively (Worksheet G03, Attachment 3). These are below a level of concern by factors of 1000 and over 14,000, respectively.

For soil injection into predominantly sandy soils (Attachment 4) or for broadcast applications of liquid formulations (Attachment 1) or granular formulations (Attachment 2), substantial risks to sensitive aquatic invertebrates are apparent based on the upper ranges of peak exposures, with hazard quotients ranging from 6 to over 80. Even at the central estimates of exposures, hazard quotients are above the level of concern for broadcast liquid formulations (HQ=8) and broadcast granular formulations (HQ=11). For these broadcast applications, the hazard quotients for longer-term exposures also exceed a level of concern at both the central and upper ranges of exposures (i.e., HQs from 1.7 to 2). Because of the very large range of sensitivities in aquatic invertebrates, however, none of the peak or longer-term hazard quotients for tolerance aquatic invertebrates exceed a level of concern.

As discussed above (Section 4.4.2.2), this risk assessment uses an extremely conservative accidental spill scenario – i.e., a spill of 0.4 lb to 40 lbs of imidacloprid into a small body of water. Over this range, the hazard quotients for sensitive aquatic invertebrates are extraordinarily high, ranging from about 500 to over 50,000. While the likelihood and plausibility of such spills may be remote, these hazard quotients clearly suggest that the greatest risk in the event of an accidental spill will be to aquatic invertebrates. As with fish and amphibians, tolerant aquatic invertebrates are not at risk in the event of an extreme spill.

4.4.3.4. Aquatic Plants – Aquatic plants are not particularly sensitive to imidacloprid and risks to aquatic plants are below a level of concern for plausible peak and longer-term concentrations of imidacloprid in water. Nonetheless, the study by Hall (1996) does suggest that at least one species of aquatic plant, *Navicula pelliculosa*, has a NOEC of 6.69 mg/L. This is lower than the NOEC values for fish, amphibians, and tolerant aquatic invertebrates (see Tables 4-4 and 4-6). Consequently, in the event of an accidental spill, hazard quotients for sensitive aquatic plants would slightly exceed a level of concern – i.e., HQs ranging from 1.1 to 3 at the upper range of exposure.

4.5. Connected Actions and Cumulative Effects

Under the NEPA, the Forest Service is required to consider the potential connected actions and cumulative effects associated with the use of imidacloprid. Connected actions related to potential impacts on risks to ecological receptors would include:

- the presence of inerts, adjuvants, impurities and metabolites in imidacloprid formulations;
- the use of irrigation in combination with application of granular formulations.

The potential impacts of metabolites and impurities has been discussed previously in this document. The risks presented here take into account the presence of these compounds. As shown in the available studies discussed previously, the toxicity of granular formulations of imidacloprid is lower among non-target plant and insect species when the recommended irrigation following application is implemented.

The cumulative effects on risks to ecological receptors associated with the use of imidacloprid could include:

- risks associated with drift from other herbicides used by others (not the Forest Service)

- physical activities such as mowing, or “acts of nature” such as drought or flooding, which could act in concert with imidacloprid to alter the growth and survival of non-target plant and animal species.
- Cumulative risk of repeated imidacloprid application (not likely given the relatively short half-life and single annual application useage)

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Table 2-1: Selected physical and chemical properties of imidacloprid.

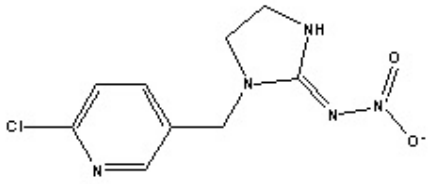
Structure	
Names and synonyms	1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine (IUPAC) (Tomlin 2004) 1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine (CAS) (Tomlin 2004) BAY NTN 33 893 (Tomlin 2004)
Appearance, ambient	Colorless crystals, with a weak characteristic odor(Tomlin 2004) Colorless, odorless crystals (Krohn and Hellpointner 2002) Lumpy light yellow powder (Yen and Wendt 1993)
Bioconcentration (Fish)	3.7 L/kg (Log BCF=0.57, reported in Meylan and Howard 2000) 0.97 to 1.5 L/kg (Ding et al. 2004)
CAS number	138261-41-3 (current), 105827-78-9 (former) (Tomlin 2004)
Density	1.54 g/cm^3 (Yen and Wendt 1993)
Foliar half-time	9.8 days (Lin 1992)
Hydrolysis	Stable at pH 5-11 (Tomlin 2004)
$K_{o/c}$	About 300 to 400 (see Appendix 7)
$K_{o/w}$ [1]	3.7 [Log Ko/w = 0.57](Tomlin 2004) 8.3 [Log Ko/w = 0.92 from HPLC retention](Nemeth-Konda et al. 2002)
Melting point	144 ˚C (Tomlin 2004)
Molecular formula	$C_9H_{10}ClN_5O_2$ (Tomlin 2004)
Molecular weight	255.7 (Tomlin 2004)
pKa	11.2 (Oliveira et al. 2000)
Sediment halftime	420 days (Meylan and Howard 2000)
Soil half-time	48 - 190 days (ExToxNet 2004)
Soil sorption, K_d	About 1 to 4 (see Appendix 7)
Smiles Notation	[O-][N+](=O)N=C1NCCN1Cc2ccc(Cl)nc2 (Tomlin 2004)
Specific Gravity	1.54 (Tomlin 2004)
Vapor pressure	4×10^{-7} mPa (20 ˚C); 9×10^{-7} mPa (25 ˚C)(Tomlin 2004) 1.5×10^{-9} mm Hg (20 ˚C) (Yen and Wendt 1993)
Water solubility	610 mg/L (Krohn and Hellpointner 2002; Tomlin 2004) 510 mg/L (Yen and Wendt 1993)

Table 2-1: Selected physical and chemical properties of imidacloprid.

[1] The $K_{o/w}$ is incorrectly cited in Graebing and Chib (2004) as 0.57.

Table 2-2: Commercial formulations of imidacloprid that may be used in Forest Service Programs for the control of ***Adelgid*** species[1].

Formulation/ Producer	Application Rates [3]	Application Type
Marathon 1% Granular/Olympic (1% a.i.)	Nurseries: 0.5 - 7 lb formulation/cu yard bulk soil	soil incorporation
	15 oz per 1000 sq ft	soil broadcast
Marathon 60 WP /Olympic (60% a.i.)	20 g per packet applied in differing amounts depending on size of pots	soil incorporation
	1 packet (20 g) per 8 to 16 inches of trunk diameter	soil injection
	1 packet per 3000 sq ft	soil broadcast/drench
Marathon II /Olympic (L, 21.4% a.i., 2 lb a.i./gallon)	19.2 to 25.6 oz/acre 50 mL/3000 sq ft	soil broadcast
	3 to 6 mL per inch of trunk diameter	soil injection
	50 mL/100 gal of water	foliar
Merit 2F/ Bayer ES (L, 21.4% (2 lb a.i. /gal)	3 to 6 mL/inch of trunk diameter	injection
	45 mL/100 gal water	foliar
Merit 2.5 G/ Bayer ES (2.5%)	Up to 4 ft in height.—use 1/4 to ½ cup 4 to 8 ft. in height —use ½ to 1 cup	soil broadcast
Merit 75 WP / Lesco and Bayer ES (75%)	1 tsp per 10 gallons of water	foliar
	1 to 2 oz per 30 cumulative inches of trunk diameter. In soil drench, use 10 gallons per 1000 sq ft	soil injection or soil drench
	1.2 to 5.6 g per 1000 sq ft	soil broadcast
Merit 75 WSP / Bayer ES (75%)	1.6 oz per 300 gal of water	foliar
	1.6 oz per 24 to 48 inches of cumulative trunk diameter. In soil drench, use 10 gallons per 1000 sq ft.	soil injection or soil drench
Provado 1.6 Flowable / Bayer CS (17.4%, 1.6 lb a.i./gal)	Trees: 4 to 8 oz/acre for Adelgids. Maximum application interval of 10 days. Maximum annual application of 0.5 lb a.i./acre. Maximum water volumes of 20 gal/acre for ground and 5 gal/acre for aerial. Poplar, Cottonwood, and Christmas Tree: 4-8 oz/acre. Maximum annual application of 0.5 lb a.i./acre for the control of adelgids on Christmas tree. Use on Christmas tree is the only labeled use specifically for adelgid control.	broadcast or directed foliar spray **Labeled for aerial application**
Imicide / Arbor Systems ©, 90% 110.7 mg/mL)	Available in 2, 3, 4, 8, 12, and 16 mL capsules. Number of capsules dependent on size of tree and severity of infestation.	Tree injection

IMA-jet / Arborjet ©, 5%)	2 mL to 8 mL depending on diameter breast height (defined as circumference of tree at chest height divided by 3).	Tree injection
Pointer / Mauget (L, 5% 6 g a.i./ 120 mL)	1mL per 4 to 6 inches of trunk circumference.	Tree injection

[1] Specimen labels from C&P Press, http://www.greenbook.net/; CDMS Label System, http://www.cdms.net/manuf/manuf.asp; U.S. EPA Label System, http://www.epa.gov/pesticides/pestlabels/index.htm , and http://www.mauget.com/mlinks/pdf/imicmsds.pdf

[2] G=Granular; WSP=Water soluble packets; WP=Wettable Powder; L=Liquid; C=Capsule

Table 2-3: Known inerts contained in commercial formulations of imidacloprid that may be used in Forest Service Programs for the control of *Adelgid* species[1].

Formulation/ Producer	Inerts Identified on MSDS [3]	Other information [4]
Marathon 1% Granular/Olympic (1% a.i.)	Quartz (CAS: 14808-60-7) 0-9%	
Marathon 60 WP /Olympic (60% a.i.)	Ingredient 1968 (Trade Secret) 3-5% Ingredient 1611 (Trade Secret) 10-20% Quartz (CAS: 14808-60-7) < 1% Ingredient 1606 (Trade Secret) 10-20% {ACGIH TWA respirable of 2 mg/m3}	
Marathon II /Olympic (L, 21.4% a.i., 2 lb a.i./gallon)	Two inerts at 1-3 % not otherwise identified.	
Merit 2F/ Bayer ES (L, 21.4% (2 lb a.i. /gal)	Glycerine (CAS No. 56-81-5)	
Merit 2.5 G/ Bayer ES (2.5%)	Quartz (CAS No. 14808-60-7) Up to 8.89% by weight.	
Merit 75 WP / Lesco and Bayer ES (75%)	Sodium aluminum silicate (CAS No. 1344-00-9) ACGIH TWA 2 mg/m3 as Al.	
Merit 75 WSP / Bayer ES (75%)	Sodium aluminum silicate (CAS No. 1344-00-9) ACGIH TWA 2 mg/m3 as Al.	Polyvinyl alcohol water soluble film Blue printing ink
Provado 1.6 Flowable / Bayer CS (17.4%, 1.6 lb a.i./gal)	Ingredients 1979 and 2035, both at 1-3%. Identities classified as trade secret.	
Imicide / Arbor Systems ©, 90% 110.7 mg/mL)	None specified.	Pylacert Oil Amber XA MX-166A (CAS Nos. 8003-22-3, 85-86-9, 81-48-1)

Pointer / Mauget (L, 5% 6 g a.i./ 120 mL)	No SARA Title III, Section 313 Toxic Chemicals. Unspecified alcohol, >94% Acrylic acid (CAS No. 79-10-7), 0.15%	Tetrahydrofurfuryl alcohol (CAS No. 97-99-4) Carbopol Resin 2984 (CAS Nos. 9003-01-4 and 79-10-7)

[1] Specimen labels from C&P Press, http://www.greenbook.net/; CDMS Label System, http://www.cdms.net/manuf/manuf.asp; U.S. EPA Label System, http://www.epa.gov/pesticides/pestlabels/index.htm , and http://www.mauget.com/mlinks/pdf/imicmsds.pdf

[2] G=Granular; WSP=Water soluble packets; WP=Wettable Powder; L=Liquid; C=Capsule

[4] http://www.pesticide.org/FOIA/imidaclo.html

Table 3-1: Toxicity data on commercial formulations of imidacloprid that may be used in Forest Service Programs for the control of *Adelgid* species[1].

Formulation [2]	Toxicity to Mammals (M: Male, F: Female) All units are formulated product
Marathon 1% Granular[3]	Rat oral LD_{50}: >4820 mg/kg Rat Dermal LD_{50}: >2000 mg/kg Rat 1-Hr Inhalation LC_{50} (Dust): >5.09 mg/L Dermal: No irritation or sensitization
Marathon 60 WP [4]	Rat oral LD_{50}: 2591 mg/kg (M), 1858 mg/kg (F) Rat Dermal LD_{50}: >2000 mg/kg Rat 4-Hr Inhalation LC_{50} (Aerosol): 2.56 mg/L (M), 2.75 mg/L (F) Dermal Irritation: Rabbit - slight irritation Dermal Sensitization: Guinea pig - none Ocular: Rabbit: Minimal and transient (24 h) irritation to conjunctiva
Marathon II (L, 21.4% a.i., 2 lb a.i./gallon)	Rat oral LD_{50}: >4870 mg/kg (M), 4143 mg/kg (F) Rat Dermal LD_{50}: >2000 mg/kg Rat 4-Hr Inhalation LC_{50} (Aerosol): >5.33 mg/L Dermal Irritation: Rabbit - no irritation Dermal Sensitization: Guinea pig - none Ocular: Rabbit: Minimal and transient (72 h) irritation to conjunctiva
Merit 2F (L, 21.4% (2 lb a.i. /gal)	Rat oral LD_{50}: >4870 mg/kg (M), 4143 mg/kg (F) Rat Dermal LD_{50}: >2000 mg/kg Rat 4-Hr Inhalation LC_{50} (Aerosol): >5.33 mg/L Dermal Irritation: Rabbit - no irritation Dermal Sensitization: Guinea pig - none Ocular: Rabbit: Mild irritation to cornea and conjunctiva clearing with 7-days.
Merit 2.5 G (2.5%)	Rat oral LD_{50}: >4820 mg/kg Rat Dermal LD_{50}: >2000 mg/kg Rat 4-Hr Inhalation LC_{50} (Dust): >5.09 mg/L Dermal Irritation: Rabbit - no irritation Dermal Sensitization: Guinea pig - none Ocular: Rabbit: Mild irritation.
Merit 75 WP (75%)	Rat oral LD_{50}: >4820 mg/kg Rat Dermal LD_{50}: >2000 mg/kg Rat 4-Hr Inhalation LC_{50} (Dust): >5.09 mg/L Dermal Irritation: Rabbit - no irritation Dermal Sensitization: Guinea pig - none Ocular: Rabbit: Mild irritation.
Merit 75 WSP(75%)	Rat oral LD_{50}: 2591 mg/kg (M), 1858 mg/kg (F) Rat Dermal LD_{50}: >2000 mg/kg Rat 4-Hr Inhalation LC_{50} (Aerosol): 2.65 mg/L (M), 2.75 mg/L (F) Dermal Irritation: Rabbit - slight irritation. Dermal Sensitization: Guinea pig - none Ocular: Rabbit: Mild irritation.

Table 3-1: Toxicity data on commercial formulations of imidacloprid that may be used in Forest Service Programs for the control of *Adelgid* species[1].

Formulation [2]	Toxicity to Mammals (M: Male, F: Female) All units are formulated product
Provado 1.6 Flowable (17.4%, 1.6 lb a.i./gal)	Rat oral LD_{50}: >4870 mg/kg (M), 4143 mg/kg (F) Rat Dermal LD_{50}: >2000 mg/kg Rat 4-Hr Inhalation LC_{50} (Aerosol): >5.33 mg/L Dermal Irritation: Rabbit - no irritation. Dermal Sensitization: Guinea pig - none Ocular: Rabbit: Minimal and transient (72 h) irritation to conjunctiva.
Imicide ©, 90% 110.7 mg/mL)	No information reported.
Pointer (L, 5% 6 g a.i./ 120 mL)	Dermal: Slightly irritating. Ocular: Substantial but transient eye irritation (NOS).

[1] Unless otherwise specified, the data are taken from MSDS sheets available at C&P Press, http://www.greenbook.net/; CDMS Label System, http://www.cdms.net/manuf/manuf.asp; U.S. EPA Label System, http://www.epa.gov/pesticides/pestlabels/index.htm , and http://www.mauget.com/mlinks/pdf/imicmsds.pdf. Also unless otherwise specified, toxicity data are on the formulation and expressed in units of formulation.

[2] G=Granular; WSP=Water soluble packets; WP=Wettable Powder; L=Liquid; C=Capsule

[3] Based on studies using 0.62% granular (ocular) or 2.5% granular (all other effects) formulations.

[4] Toxicity values extrapolated from an unspecified formulation containing a higher proportion of the a.i.

Table 3-2: Chemical and site parameters used in GLEAMS modeling for imidacloprid.

Chemical Specific Parameters

Parameter	Clay	Loam	Sand	Comment/ Reference
Halftimes (days)				
Aquatic Sediment		27		Fritz and Hellpointner 1991
Foliar		10		Note 1
Soil		40		Note 2
Water		22		Note 3
$K_{o/c}$, mL/g	779	296	203	Note 4
K_d, mL/g	11.3	3.45	1.18	Note 4
Water Solubility, mg/L		610		Krohn and Hellpointner 2002; Tomlin 2004
Foliar wash-off fraction		0.5		Note 5
Fraction applied to foliage		0.5/0.01/0		Note 6

Other Model Parameters: See SERA 2004 for other standard model parameters. All runs based on short leaf conifer forest (GLEAMS FOREST Code 2). See text for the discussion of site characteristics.

Note 1	Based on reported halftime of 9.8 days on turf from Lin 1992a,c. Much shorter halftimes (about 1 day) have been reported (Lin 1992d).
Note 2	Based on Sarkar et al. 2001 reporting an average of 39 days with a range of 27.8 to 44.9 days for Conifer formulation and 40.7 days with range of 35.8 to 46.3 days for Gaucho formulation.
Note 3	Imidacloprid is stable to hydrolysis but aqueous photolysis is rapid, with experimental halftimes of 4.2 hours (Anderson 1991) and 1.2 hours (Moza et al. 1998). The 4.2 hour value is used because it follows EPA guidelines. Based on the approach used by U.S. EPA/OPP 2001a, the effective photolysis halftime is taken as 124 times longer than the experimental value to account for light attenuation. 124×4.2 hours = 21.7 days. This is likely to be highly variable and site specific.
Note 4	The soil binding characteristics of imidacloprid are complex. See Appendix 8 for summary of experimental data and text for discussion. Values from sand and clay from Oliveira et al. 2000 (ARS/USDA). Values for loam from Williams et al. 1992a, MRID 42520801.
Note 5	No data available. This is not a sensitive parameter.

Note 6	No data available. A value of 0.5 used for foliar as a default. For granular applications, foliar application will be negligible. For soil injection, the fraction applied to foliage is set to zero and the depth of incorporation is set to 15 cm (about 6 inches).

Table 3-3: Estimated environmental concentrations (µg/L or ppb) of imidacloprid in a stream based on GLEAMS modeling normalized for an application rate of 1 lb/acre[1].

Scenario		Peak	Long-Term Average
Liquid Formulation			
	Clay	0.4 to 51	0.009 to 0.2
	Loam	0.01 to 7	<0.001 to 0.02
	Sand	<0.001 to 4	<0.001 to 0.1
Granular Formulation			
	Clay	0.6 to 71	0.01 to 0.3
	Loam	0.02 to 9	<0.001 to 0.03
	Sand	<0.001 to 5	<0.001 to 0.2
Soil Injection			
	Clay	No losses at any rainfall rate	
	Loam	<0.001 at all rainfall rates	
	Sand	<0.001 to 3	<0.001 to 0.08

[1] See Table 1 in Appendices 10 to 12 for details and text for discussion. No losses modeled at annual rainfall rates of 5 or 10 inches.

Table 3-4: Estimated environmental concentrations (µg/L or ppb) of imidacloprid in a pond based on GLEAMS modeling normalized for an application rate of 1 lb/acre[1].

Scenario		Peak	Long-Term Average
Liquid Formulation			
	Clay	0.6 to 37	0.2 to 1
	Loam	<0.001 to 5	<0.001 to 0.1
	Sand	<0.001 to 3	<0.001 to 0.3
Granular Formulation			
	Clay	0.9 to 52	0.2 to 1
	Loam	0.01 to 7	0.001 to 0.2
	Sand	<0.001 to 5	<0.001 to 0.4
Soil Injection			
	Clay	No losses at any rainfall rate	
	Loam	<0.001 at all rainfall rates	
	Sand	<0.001 to 3	<0.001 to 0.3

[1] See Table 2 in Appendices 10 to 12 for details and text for discussion. No losses modeled at annual rainfall rates of 5 or 10 inches.

Table 3-5: Water contamination rates (mg/L per lb/acre) in surface water used in this risk assessment (see Section 3.2.3.4.6 for discussion).

	Peak Concentration (ppm or mg/L)	Longer Term Concentration (ppm or mg/L)
Liquid Formulations	**Clay and Loam Soils**	
Central	0.007	0.0007
Lower	0.0005	0.0001
Upper	0.05	0.001
Granular Formulations	**Clay and Loam Soils**	
Central	0.01	0.001
Lower	0.0006	0.0002
Upper	0.07	0.001
Soil Injection	**Clay and Loam Soils**	
Central	0	0
Lower	0	0
Upper	0.000001	0.00000003
Broadcast or Soil Injection	**Sand**	
Central	0.0001	0.00004
Lower	0.00006	0.00001
Upper	0.005	0.0001

Table 4-1: Overview of imidacloprid toxicity to birds.

Organism	Endpoint	Toxicity Value [a]	Reference
Bobwhite quail	acute NOAEL	25 mg/kg bw	Toll 1990a
	acute LOAEC (mortality)	69 ppm	Toll 1990b
	chronic LOAEC (hatchling mortality)	36 ppm ~2.25 mg/kg /day [b]	Toll 1991b
Mallard duck	acute NOAEC (mortality)	>5000 ppm	Toll 1991a
	chronic NOAEC (eggs laid, viability, hatchling survival and growth)	125 ppm	Toll 1991c
	chronic NOAEC (reproduction, growth, survival, eggshell thickness/strength)	128 ppm	Stafford 1992
	chronic LOAEC (eggshell thickness and strength)	250 ppm	Stafford 1992
	chronic NOAEC (eggshell thickness and strength, mortality, clinical signs)	47 ppm (highest dose tested)	Hancock 1994b
Canary	acute LOAEL (clinical signs of neurotoxicity)	10 mg/kg bw	Grau 1994b
House Sparrow	acute NOAEL (clinical signs of neurotoxicity)	3 mg/kg bw	Stafford 1991
Japanese Quail	acute NOAEL (clinical signs of neurotoxicity)	3.1 mg/kg bw	Grau 1988b
	acute LOAEC (mortality, apathy, narcosis)	313 ppm diet	Grau 1994a
Pigeon	acute LOAEL (clinical signs: apathy, cramps, prone position)	12.5 mg/kg bw	Grau 1994b
Ringed turtle dove	acute LOAEC (reduced body weight, food consumption, clinical signs: ataxia, fluffed feathers, hypoactivity)	228 ppm diet	Hancock 1994a

American robin, cardinal, gray catbird, blue jay, brown thrasher, northern mockingbird, rufus-sided towhee	acute field application NOAEL for Merit 0.62% granular applied to golf course turf; no effect on survival or percent mortality between control and treated sites.	0.5 lb a.i./acre	Toll and Fischer 1993

[a] all values are in terms of active ingredient

[b] 36 mg/kg diet x 0.018 kg diet/day x a/0.288 kg bird = 2.25 mg imidacloprid/kg bird/day; based on experimental data and mean female body weight for birds fed 36 ppm imidacloprid in the diet.

Table 4-2: Overview of imidacloprid toxicity values in bees and earthworms

Organism	Endpoint	Toxicity Value[a]	Reference
Honey Bee	acute oral LOAEL	1.5 ng/bee	Cole 1990
	acute contact LOAEL	25 ng/bee	Cole 1990
	acute oral NOAEL	1.5 ng/bee	Nauen et al. 2001
	acute oral NOAEL	1.2 ng/bee	Schmuck et al. 2001
	chronic NOAEC (mortality and foraging activity)	24 ug/kg = 0.97 ng/bee	Decourtye et al. 2003; 2004
	chronic NOAEC	20 ug/kg	Schmuck et al. 2001
	chronic LOAEC	8 ng/bee	Dechaume Moncharmont 2003
	sub-lethal laboratory conditioned behavioral effects		
	NOAEC olfactory learning	6 ug/kg ~0.24 ng/bee	Decourtye et al. 2003
	LOAEL proboscis extension reflex (PER) habituation	1 ng/bee	Guez et al. 2001;
		24 ug/kg ~ 0.97 ng/bee	Decourtye et al. 2003;
		1.25 ng/bee	Lambin et al. 2001
Bumble bee	chronic field NOAEC for Merit 75	0.336 kg a.i./ha	Gels et al. 2002
	chronic field NOAEC for Merit 0.5G	0.336 kg a.i./ha	Gels et al. 2002
Earth worm (*Eisenia foetida*)	NOAEC sperm deformity	0.1 mg/kg soil	Luo et al.1999

	field LOAEC (transient decrease in abundance; gone 36-40 days post-application)	0.45 kg a.i/ha (Merit 75 WP); 0.34 kg a.i./ha, spray + irrigation (Merit 0.5G, drop spreader + irrigation)	Kunkel et al. 1999

[a] All concentrations are expressed in terms of active ingredient.

Table 4-3: Overview of imidacloprid toxicity to beneficial predatory arthropods.

Formulation	Species	Application Rate/Method	Observed Effect	Reference
Technical Grade	Convergent lady beetle (*Hippondamia convergens*)	LD_{50} test/topical application to insect	72-hour LD50 = 0.4 mg/kg (dose based on insect body weight)	Kaakeh et al. 1996
Not Specified	Beetle *(Harpalus pennsylvanicus*)	25%, 50% and 100% 0.336 kg/ha to plots/ spray	LOAEC = 0.084 kg ai/ha, incapacitation within 4 hours, recovery within 4 days for 85% of beetles	Kunkel et al. 2001
		dog pellets sprayed with 25%, 50% and 100% 0.336 kg/ha	As above for transient intoxication. Also, NOAEC, fecundity, timing egg hatching = 0.336 kg ai/ha	Kunkel et al. 2001
Admire (240 g/L EC)	predacious Mirid bug (*Hyaliodes vitripennis*)	geometric progression based on label rate of 0.0312 g a.i./L; direct spray to insect/leaves/cage	24-hour Nymph LC_{50}: 0.0023 g a.i./L; Adult LC_{50} : 0.0011 g a.i./L	Bostanian et al 2001

Table 4-3: Overview of imidacloprid toxicity to beneficial predatory arthropods.

Formulation	Species	Application Rate/Method	Observed Effect	Reference
Admire 2F	Vedalia beetle (*Rodolia cardinalis*)	72-hour exposure to cottony cushion scale larvae raised on plants growing in imidacloprid-treated soil (0.15 ml, Admire 2F)	LOAEC: significantly reduced mean percentage of adult beetles and progeny with respect to controls on day 22 post-exposure, but not on days 43-155 post-exposure. Significantly reduced number of 2nd instar larvae surviving to adulthood (0 - 24.44% on days 8 - 29 after treatment; 51.11 - 66.67 on days 57 through 141 after treatment); recovery to control percentages on 169 days post-treatment.	Grafton-Cardwell and Gu 2003
Confidor 350 SC	parasitoid Hymenopteran (*Trichogramma nr. Brassicae*)	field application rate = 5.25 g a.i./100 L, single direct application to adults	100% mortality after 3 hours	Hewa-Kapuge et al. 2003
Confidor 350 SC	parasitoid Hymenopteran (*Trichogramma nr. Brassicae*)	potted tomato plants sprayed to runoff at label rate; wasps exposed after spray	mortality LOAEC = 100 g a.i./100L, on day of spray only. No effects on later days. No effects on reproduction/growth. No effects when parasite eggs dipped in formula at 100 g ai/L	Hewa-Kapuge et al. 2003

Table 4-3: Overview of imidacloprid toxicity to beneficial predatory arthropods.

Formulation	Species	Application Rate/Method	Observed Effect	Reference
	Phytoseiid mite (*Amblyseius victoriensis*)	sprayed on grape leaf discs at field rate to control aphids (5.25 g a.i./100L or 0.0053% a.i.) and 10X this rate	NOAEC: 0.0525 g/L LOAEC (mortality): 0.525 g/L	James 1997
		apricot orchard study. Spray at field rate of 5.25 g a.i./100 L	LOAEC: significant transient reduction in population 4 weeks after exposure. However, population rebounded to exceed control values 9-12 weeks after exposure	James 1997
Confidor 200 SL	Predatory bug (*Orius laevigatus*)	72-hour acute ingestion toxicity test with Confidor 200 SL (Imidacloprid a.i.), 8 concentrations	Nymph LC_{50}: 1.1 mg a.i./L Adult LC_{50}: 2.1 mg a.i./L	Delbecke et al. 1997
		72-hour residual contact test with Confidor 200 SL (Imidacloprid a.i.), 5 concentrations	Nymph LC_{50}: 0.04 mg a.i./L Adult LC_{50}: 0.3 mg a.i./L	

Table 4-3: Overview of imidacloprid toxicity to beneficial predatory arthropods.

Formulation	Species	Application Rate/Method	Observed Effect	Reference
Confidor 20LS (20% a.i.)	Predatory bug nymphs (*Dicyphus tamaninii*) and (*Macrolophus caliginosus*)	0.5 ml/L (maximum recommended rate)/tomato plants sprayed until runoff; bugs exposed at various intervals after spray	LOAEC: *D. tamaninii* was more sensitive, with mortality ranging from 33.7% 24 hours after exposure to 1-day residues, to 91.9 % 7 days after exposure to 1-day residues. Percent mortality declined with increasing residue time, with 2 to 26.0% mortality at 24 hours and 7-days, respectively, after exposure to 30 day residues.	Figuls et al. 1999
Marathon 60 WP	*Euonymus* scale parasitoid Hymenopteran (*Encarsia citrina*)	Soil drench at 0.33 g/500 ml water; foliar application at 0.15 g/500 ml of water	No effect on parasitoid infectivity	Rebek and Sadof 2003

Table 4-3: Overview of imidacloprid toxicity to beneficial predatory arthropods.

Formulation	Species	Application Rate/Method	Observed Effect	Reference
Merit 0.5 G	Predatory arthropods assessed in field study: ants, carabids, spiders, and staphylinids	0.336 kg a.i./ha, drop spreader with irrigation; study replicated in 1996 and 1997	LOAEC: reduced the abundance of hister beetles and predatory larvae across all sample dates in 1996 but not in 1997; reduced scavenging rates on fresh-frozen black cutworms during the first week after treatment, but scavenging activity returned to normal with respect to controls 2-4 weeks post-treatment. There was no difference between controls and imidacloprid-treated plots with respect to scavenging of black cutworm eggs or Japanese beetle eggs. Ants were the primary predators	Kunkel et al. 1999
	Predatory ants	0.34 kg a.i./ha by drop spreader or hand broadcast to plots of turf-grass, with irrigation	NOAEC: abundance of ants and absence of prey (Japanese beetles and white grubs).	Zenger and Gibb 2001
Provado 1.6F	parasitoid Ichneumonidae Hymenopteran (*Diadegma insulare*)	leaf dip equivalent to various field application rates in units of mg a.i./ml at spray volume of 240 L/ha.	24-hour LC_{50}: 0.002 mg a.i./ml = 0.00048 kg a.i./ha (2 mg/L x 240 L/ha x 1E-6 kg/mg = 0.00048 kg/ha)	Hill and Foster 2000

Table 4-3: Overview of imidacloprid toxicity to beneficial predatory arthropods.

Formulation	Species	Application Rate/Method	Observed Effect	Reference
	Insidious flower bug (*Orius insidiosus*)	*Helicoverpa zea* eggs sprayed at 0.052 kg a.i. imidacloprid/ha	NOAEC: egg consumption and fecundity LOAEC: mortality	Elzen 2001
	Big-eyed bug (*Geocoris punctipes*)	*Helicoverpa zea* eggs sprayed at 0.052 kg a.i. imidacloprid/ha	LOAEC: mortality and reduced egg consumption	Elzen 2001
	Vedalia beetle (*Rodolia cardinalis*)	0.56 kg a.i./ha ; soil drench 0.14 kg a.i/ha; foliar spray	72-hour NOAEC adult survival and reproduction. LOAEC larval survival: all dead within 3 days. 72-hour LOAEC for adult and larval survival: significantly reduced adult survival and progeny per female 26 days after treatment; all larvae died within 8 days of exposure to treated foliage	Grafton-Cardwell and Gu 2003

Table 4-3: Overview of imidacloprid toxicity to beneficial predatory arthropods.

Formulation	Species	Application Rate/Method	Observed Effect	Reference
	Vedalia beetle (*Rodolia cardinalis*	72-hour exposure to cottony cushion scale larvae raised on plants sprayed to runoff with 0.2 ml Provado 1.6F	LOAEC: significantly reduced mean percentage of adult beetles and progeny with respect to controls on day 20 and 41 post-exposure, but not thereafter up to day 182. Significantly reduced percentage of larvae reaching adulthood for beetles exposed to treated scale insects and leaves on days 6 through 27 after treatment only	Grafton-Cardwell and Gu 2003
Provado 2F	ectoparasitoid Hymenopteran (*Colpochypeus florus*)	"48 ppm or amount/100 gallons"/ direct spray (100% application rate for controlling leafhopper in apple trees) and exposure to residues	LOAEC: 86% mortality in 48-hours following direct spray; no mortality if insects are exposed to leaves after they are sprayed.	Brunner et al. 2001
	ectoparasitoid Hymenopteran (*Trichogramma platneri*)	48 ppm or amount/100 gallons"/ direct spray (100% application rate for controlling leafhopper in apple trees)	100% mortality in 48 hours	Brunner et al. 2001

Table 4-4: Overview of imidacloprid toxicity values in aquatic invertebrates.

Organism	Endpoint	Toxicity Value[a]	Reference
Water Flea (*Daphnia magna*)	48-hour LC_{50}	10.44 mg/L	Song et al. 1997; Song and Brown 1998
	48-hour EC_{50}	85 mg/L	Young and Hicks 1990
	48-hour NOAEC (immobility)	42 mg/L	Young and Hicks 1990
	21-day EC_{50} (immobilization)	>7.3 mg/L	Young and Blake 1990
	21-day NOAEC (immobilization)	1.8 mg/L	Young and Blake1990
	21-Day LOAEC (immobility)	3.6 mg/L	Young and Blake 1990
Amphipod Crustacean (*Hyalella azteca*)	96-hour LC_{50}	0.526 mg/L	England and Bucksath 1991
	96-hour NOAEC	0.00035 mg/L	England and Bucksath 1991
Midge (*Chironomus tetrans*)	96-hour LC_{50}	0.0105 mg/L	Gagliano 1991
	96-hour NOAEC	0.00124 mg/L	Gagliano 1991
	10-day LC_{50}	0.00317 mg/L	Gagliano 1991
	10-day NOAEC (growth)	0.00067 mg/L	Gagliano 1991
Saltwater Mysid (*Mysidopsis bahia*)	96-hour LC_{50}	0.0377 mg/L 0.0341 mg/L	Ward 1990b
	96-hour LC_{50} for NTN33893 240 FS formulation	0.036 mg a.i./L	Lintott 1992
	NOAEC (mortality, loss of equilibrium)	0.0133 mg/L	Ward 1990b
	NOAEC (mortality) for NTN33893 240 FS formulation	0.021 mg a.i./L	Lintott 1992
	chronic NOAEC (growth and reproductive success)	0.000163 mg/L	Ward 1991

	chronic LOAEC (growth)	0.000326 mg/L	Ward 1991
Eastern Oyster (*Crassostrea virginica*)	96-hour NOAEC (survival, shell growth)	145 mg/L	Wheat and Ward 1991
Multiple species, freshwater microcosm study	NOAEC (total macroinvertebrates and taxonomic richness)	0.002 mg/L	Moring et al. 1992
Multiple species, freshwater microcosm study	LOAEC (transient amphipod sensitivity)	0.002 mg/L	Moring et al. 1992

[a] All concentrations are expressed in terms of active ingredient.

Table 4-5: Soil contamination rates (mg/L per lb/acre) for the top 12 inches of soil that are used in this risk assessment (see Section 4.2.4. for discussion).

		Peak Concentration (ppm or mg/kg soil)	Longer Term Concentration (ppm or mg/kg soil)
Liquid Formulations	**Clay and Loam Soils**		
	Central	0.17	0.04
	Lower	0.13	0.0006
	Upper	0.19	0.05
Granular Formulations	**Clay and Loam Soils**		
	Central	0.25	0.05
	Lower	0.23	0.009
	Upper	0.26	0.06
Soil Injection	**Clay and Loam Soils**		
	Central	0.25	0.05
	Lower	0.23	0.04
	Upper	0.27	0.07
Broadcast or Soil Injection	**Sand**		
	Central	0.23	0.03
	Lower	0.13	0.008
	Upper	0.23	0.05

Table 4-6: Summary of imidacloprid toxicity values used in the ecological risk assessment to characterize risk to nontarget organisms (*see Table 4-7 for values for terrestrial invertebrates*).

Organism	Endpoint	Toxicity Value [a]	Reference
Mammals (rats)	Acute LOAEL, females, locomotor activity, 42 mg/kg bw	14 mg/kg [b]	Sheets 1994a,b: EPA 2003
	Chronic NOAEL, males, thyroid changes, reduced body weight	5.7 mg/kg/day [c]	Eiben and Kaliner 1991; Eiben 1991
Birds (house sparrow)	Acute NOAEL	3 mg/kg	Stafford 1991
	Chronic LOAEC, hatchling body weight, 36 ppm diet (bobwhite quail); acute NOAEL, 3 mg/kg (house sparrow)	0.3 mg/kg/day [d]	Toll 1991b; Stafford 1991
Honey Bee	Acute NOAEL, 1.2 ng/bee	[e]0.013 mg/kg bw	Schmuck et al. 2001
Fish, Acute			
Sensitive (bluegill)	96-hour NOAEC	25 mg/L	Bowman and Bucksath 1990a
Tolerant (rainbow trout)	96-hour NOAEC	50 mg/L	Grau 1988a
Fish, Chronic			
Sensitive/tolerant	NOAEC(early life stage test)	9.8 mg/L	Cohle and Bucksath 199; Gagliano 1992
Amphibians, Acute			
Sensitive (*Rana linocharis*)	96-hour NOAEC	30 mg/L	Feng et al. 2004
Tolerant (*Rana hallowell*)	96-hour NOAEC	101.2 mg/L	Feng et al. 2004
Amphibians, Chronic			
Sensitive (*Pseudacris triseriata*)	Developmental NOAEC	17.5-20 mg/L	Julian and Howard 1999
Tolerant (*Bufo americanus)*	Developmental NOAEC	88-110 mg/L	Julian and Howard 1999
Aquatic Invertebrates, Acute			
Sensitive (*Hyalella azteca*)	96-hour NOAEC	0.00035 mg/L	England and Bucksath 1991
Tolerant (Eastern oyster)	96-hour NOAEC (survival, shell growth)	145 mg/L	Wheat and Ward 1991
Aquatic Invertebrates, Chronic			
Sensitive (*Mysidopsis bahia*)	chronic NOAEC, growth reproductive success	0.000163 mg/L	Ward 1991
Tolerant (*Daphnia magna)*	21-day NOAEC, immobility	1.8 mg/L	Young and Blake 1990

Table 4-6: Summary of imidacloprid toxicity values used in the ecological risk assessment to characterize risk to nontarget organisms (*see Table 4-7 for values for terrestrial invertebrates*).

Organism	Endpoint	Toxicity Value [a]	Reference
Aquatic Algae			
Sensitive/tolerant (*Scenedesmus subspicatus*)	96-hour NOAEC, biomass and growth	10 mg/L (test limits)	Heinbach 1989
Sensitive (Navicula pelliculosa)	4-day NOAEC	6.69 mg a.i./L	Hall 1996
Tolerant (*Selanastrum capricornutum*)	5-day NOAEC, biomass and growth	119 mg/L (test limits)	Gagliano and Bowers 1991

[a] all values expressed as active ingredient.
[b] LOAEL of 42 mg/kg ÷ 3 = NOAEL of 14 mg/kg. EPA uses this value in deriving the acute RfD of 0.14 mg/kg for imidacloprid.
[c] This value is the basis for EPA's chronic RfD of 0.057 mg/kg/day for imidacloprid.
[d] equivalent to bobwhite quail LOAEL of 2.25 mg/kg/day ÷6.75 = 0.3 mg/kg/day; also equivalent to house sparrow acute NOAEL of 3 mg/kg ÷10 = chronic NOAEL of 0.3 mg/kg/day
[e] 1.2 ng/bee ÷ 9.3E-5 kg/bee x 1E-6 mg/ng = 0.013 mg/kg bw.

Table 4-7: Summary of toxicity values for terrestrial invertebrates.

Organism	Endpoint	Toxicity Value [a]	Reference
Bees			
Honey Bee	Chronic NOAEC, 11-day dietary, 24 ug/kg (imidacloprid in a 500 g/L sucrose solution)	0.010 mg/kg/day [b]	Decourtye et al. 2003
Bumble Bee	chronic field NOAEC (Merit 75 spray or Merit 0.5 G mixed with sand and hand-applied, both with irrigation)	0.336 kg /ha	Gels et al. 2002
Beneficial predator, direct spray			
sensitive (Bug: *Orius laevigatus* nymphs)	contact LC_{50}, nymphs; spray application	0.04 mg /L	Delbecke et al. 1997
Beneficial predator, foliar spray			
sensitive (parasitic wasp: *Diadegma insulare*)	24-hour LC_{50}, 0.002 mg/ml	0.00048 kg./ha [c]	Hill and Foster 2000
tolerant (Vedalia beetle)	72-hour LOAEC adult/larval survival	0.14 kg /ha	Grafton-Cardwell and Gu 2003
Beneficial predator, soil drench			
sensitive/tolerant (Vedalia beetle)	72-hour NOAEC: adult/larval survival	0.56 kg/ha	Grafton-Cardwell and Gu 2003
Beneficial predator, granular application, drop spreader			
sensitive (hister beetles and predatory larvae)	LOAEC: transient reduction in abundance	0.336 kg /ha	Kunkel et al. 1999
tolerant (predatory ants)	NOAEC: abundance and absence of prey	0.34 kg /ha	Zenger and Gibb 2001
Earthworm	field LOAEC (transient decrease in abundance; gone 36-40 days post-application)	0.34 kg a.i/ha (Merit 75 WP spray + irrigation ; Merit 0.5G, drop spreader + irrigation); 0.45 kg a.i./ha (Merit 75WP spray + irrigation)	Kunkel et al. 1999

Table 4-7: Summary of toxicity values for terrestrial invertebrates.

Organism	Endpoint	Toxicity Value [a]	Reference
	NOAEC sperm deformity	0.1 mg/kg soil	Luo et al.1999
Terrestrial Fungi	LOAEC (growth inhibition)	10 ppm soil	Tu 1995

[a] All values expressed as active ingredient.
[b] Assumes: density of a 500 g/L sucrose solution = 1227 kg/m^3; 1E-3 m3/L; bees consume 3.3E-7 L/day; bees weigh 9.3E-5 kg. 24 ug/kg x 1227 kg/m3 x 0.001 m3/L x 3.3E-5 L/bee x 0.001 mg/ug ÷ 9.3E-5 kg/bee = 0.010 mg/kg/day.
[c] 0.002 mg/ml = 2 mg/l; at the experimental spray rate of 240 L/ha, 2 mg/Lx 240 L/ha = 480 mg/ha or 0.00048 kg/ha.

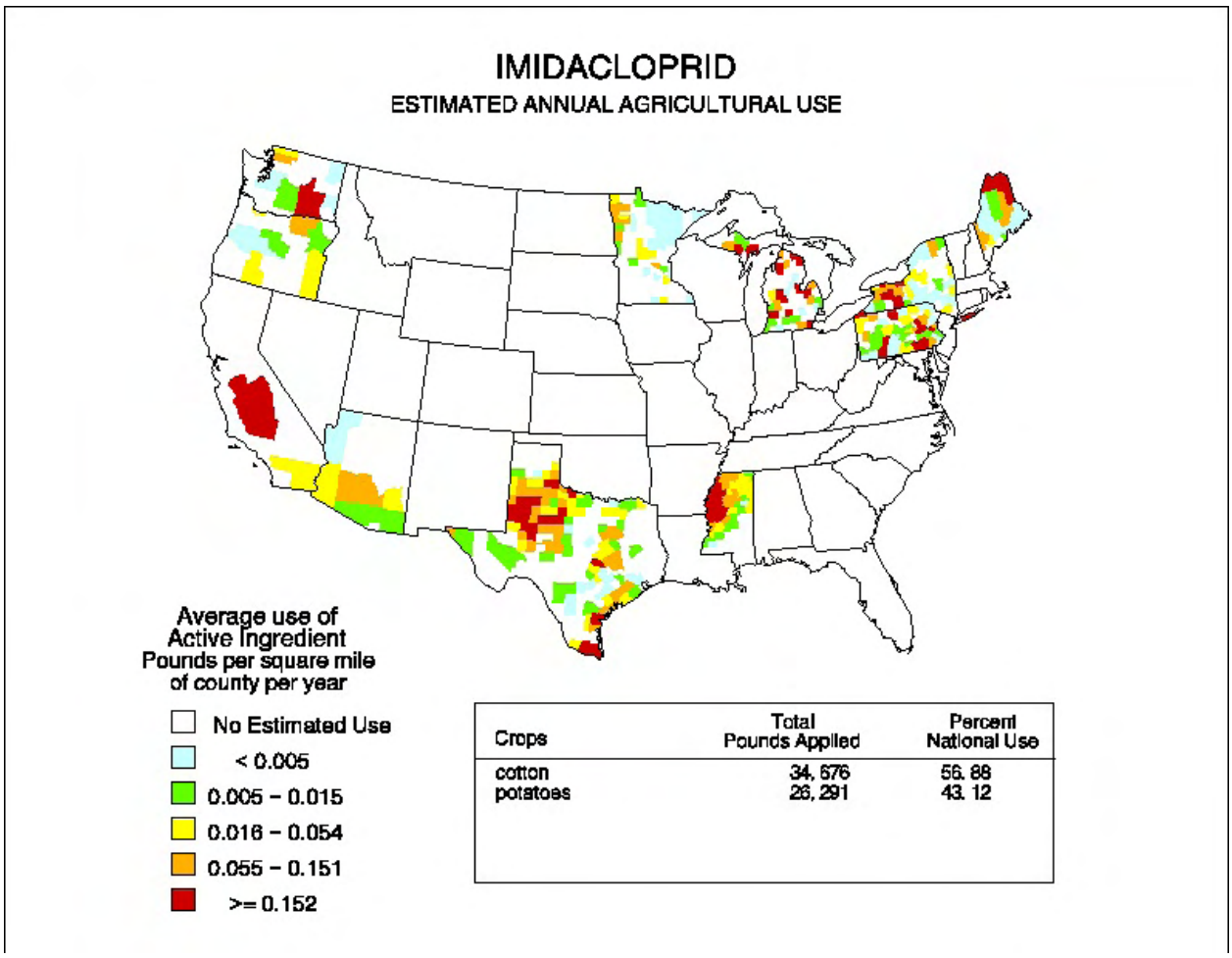


Figure 2-1: Agricultural uses of imidacloprid (USGS 1998a)

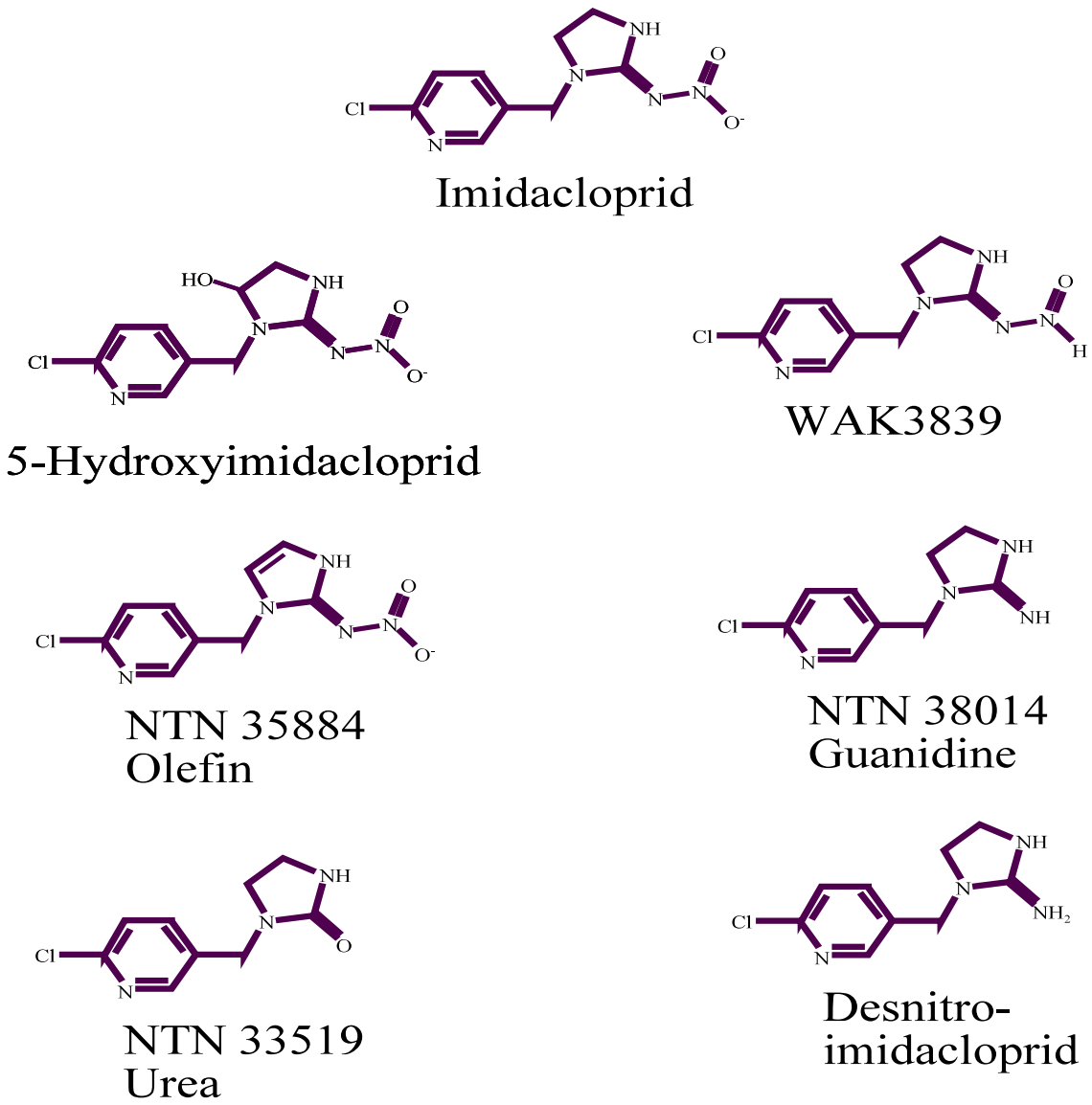


Figure 3-1: Structure of imidacloprid and related compounds.

APPENDICES

Appendix 1: Acute toxicity to experimental mammals

Appendix 2: Longer-term toxicity studies in mammals

Appendix 3: Toxicity to birds after oral administration

Appendix 4: Toxicity to non-target terrestrial invertebrates

Appendix 5: Toxicity to fish

Appendix 6: Toxicity to aquatic invertebrates

Appendix 7: Toxicity to aquatic plants

Appendix 8: Physical chemical properties and laboratory studies on environmental fate

Appendix 9: Field or field simulation studies on environmental fate

Appendix 10: GLEAMS modeling, 2 acre plot, liquid formulation

Appendix 11: GLEAMS modeling, 2 acre plot, granular formulation

Appendix 12: GLEAMS modeling, 1 acre plot, soil injection

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
ORAL			
Rats, Gavage			
Rat, Sprague-Dawley (Sas:CD (SD)BR), 5 male (8 weeks old) and 5 female (10 weeks old)	Single gavage dose of 4820 mg/kg (analytically determined) BAY NTN 33893 2.5% Granular in deionized water (10 ml/kg); formulation is 2.6% active ingredient (a.i.)	No death. No clinical signs. No gross lesions observed at necropsy. All rats gained body weight. NOAEL >4820 mg formulation/kg body weight LD50 >4820 mg formulation/kg body weight	Sheets 1990a MRID 42055324
Rat, Wistar (Bor: WSIWSPF-Cpb), 5 male (7-8 weeks old, 167-187 g), and 5 female (10-12 weeks old, 168 - 194 g) per dose	LD_{50}, NTN 33893 Technical (94.2% a.i.) By gavage at doses of 50, 100, 250, 315, 400, 450, 500 and 1800 mg/kg body weight. Vehicle = 2% v/v Cremophor EL in demineralized water (10 ml/kg)	LD_{50} = 424 mg/kg body weight (males) LD_{50} >450; <475 mg/kg body weight (females) NOAEL (mortality) = 315 mg/kg body weight; mortality in both sexes at doses = 400 mg/kg body weight and higher. NOAEL (clinical signs) = 50 mg/kg. Apathy, labored breathing, accelerated breathing, decreased mobility, staggering gait, blepharophemosis, trembling, spasms seen after dosing at doses of 100 mg/kg body weight and higher, but reversible within 2 - 6 days.	Bomann 1989b MRID 420553331

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Rat, Sprague-Dawley (Sas: CD (SD)BR), 12/sex/dose for neurobehavioral evaluation and 6/sex/dose (satellite group) evaluated for clinical pathology, 12 weeks old	Acute oral neurotoxicity screening study: single gavage dose of NTN 33893 Technical (97.6 - 98.8% a.i.) At confirmed doses of 0 (vehicle), 42, 151 and 307 mg/kg body weight. Vehicle 0.5% methylcellulose and 0.4% Tween (w/v) in deionized water (10 ml/kg)	Mortality in 4/18 high-dose males and 10/18 high-dose females within 24 hours of exposure. Dose-related increase in clincal signs (males $\underline{>}$ 151 mg/kg and 307 mg/kg females). All clincal signs and neurobehavioral effects are attributed to acute cholinergic toxicity. Recovery from all signs and neurobehavioral effects within 7 days. NOAEL (neurofunctional battery): 42 mg/kg **LOAEL (females: decreased measures of motor and locomotor activity): 42 mg/kg**; NOAEL (clincal chemistry): 42 mg/kg: decreased serum triglycerides; decreased serum potassium and cholesterol for females; decreased serum ALT; NOAEL (body weight, organ weights, gross and microscopic pathology): 307 mg/kg	Sheets 1994a MRID 43170301 Sheets 1994b MRID 43285801 (Supplemental transmission)
Rat, Sprague-Dawley (Sas: CD (SD)BR), 12 females/dose for neurobehavioral evaluation , 12 weeks old	Supplemental study: single gavage dose of NTN 33893 technical (97.6 - 98.8% a.i.) at confirmed doses of 0 (vehicle) and 20 mg/kg body weight. Vehicle 0.5% methylcellulose and 0.4% Tween (w/v) in deionized water (10 ml/kg)	**NOAEL: 20 mg/kg body weight**. No mortality, clinical signs, effects on body weight. No neurological effects as tested in the first study above.	

Note: The above LOAEL of 42 mg/kg from Sheets 1994 is the basis for EPA's acute RfD for imidacloprid

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Rat, Sprague-Dawley, (Sas: CD (SD) BR) 5/sex/dose, approximately 11 weeks old.	$LD_{50,}$ BAY NTN 33893 75 WP-WS (76.1% a.i.) by gavage in Cremophor EL in deionized water (10 ml/kg) at doses of 1063, 2180 and 3170 mg/kg body weight. for males; and doses of 1063, 2180, 2750 and 3170 mg/kg body weight for females	LD_{50} = 2591 mg/kg, males LD_{50} = 1858 mg/kg, females LOAEL = 1063 mg/kg 20% mortality, both sexes at 1063 mg BAY NTN 33893/kg (lowest dose). Dose- related decrease in body weight gain by day 14; treatment related toxicity (tremors, labored breathing, diarrhea, increased reactivity, decreased reactivity, eyes partially shut, stained fur, salivation, lacrimation etc.) resolved (recovery) in a dose-related manner by day 14.	Sheets and Phillips 1991a MRID 42256312
Rat, Sprague-Dawley, (Sas: CD (SD) BR) 5/sex/dose	LD_{50}, BAY NTN 33893 240 F.S (23.1% a.i.) By gavage in Cremophor EL in deionized water (5 ml/kg) at doses of 1030, 2100, 3595 and 4870 mg/kg body weight for males; 2100, 3595 and 4870 mg/kg body weight for females	LD_{50} > 4870 mg/kg, males LD_{50} = 4143 mg/kg, females NOAEL= 1030 mg/kg, males; LOAEL= 2100 mg/kg, females Dose-related increase in mortality for females but not males. Lacrimation, decreased motor activity, tremors, convulsions seen on day of dosing but resolved in survivors by day 2. Dose-related decrease in body weight gain days 0 to 7, but resolved days 7 - 14 for both males and females. No gross treatment-related lesions other than lacrimation in one female.	Sheets 1990f MRID 42256313

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Rat, Sprague-Dawley (Sas: CD/SD/BR), 5 or 6 male (179 - 260 g), and 6 female (171-209 g) per dose, 8-10 weeks old	LD_{50}, single gavage administration of Imidacloprid (BAY T-7391) 10% Pour On (9.88 - 10.01% w/v a.i.) In PEG 400/deionized water (1:1 v/v) at analytically confirmed doses of 0, 495, 1020, 1430 (5 males treated only), 1910 or 2620 mg/kg body weight	LD_{50} = 1943 mg/kg bw (95% CI not calculable), males; LD_{50} = 1732 mg/kg bw (95% CI = 1416 - 2147 mg/kg), females LOAEL (clinical signs) = 495 mg/kg: number of rats affected and types of signs are dose-related; signs included hypoactivity, increased reactivity, labored breathing, locomotor incoordination, tremors and oral and nasal staining. Convulsions were seen in one rat at the highest dose. Signs resolved by day 3.	Warren 1995a MRID 43679601
Rat, Sprague-Dawley, 5 female	Acute oral toxicity up and down procedure. Single gavage dose of 2000 mg Permatek IM 30 (31g/L a.i.)/kg body weight, administered as supplied.	No mortality, clinical signs or gross findings at necropsy. Body weight gain was reported to be satisfactory, although no controls were used. LD_{50} > 2000 mg/kg bw	Pritchard and Donald 2004a MRID 46290903
Rat, SD (Crj:CD), 5 male, 5 female per dose, 7 weeks old, fasted, non-fasted	LD_{50}, NTN 37571 (nitrosimine metabolite; % a.i. not reported) by gavage in DMSO and polyethylene glycol 400 (10 ml/kg) at doses of 150, 300, and 600 mg/kg body weight (fasted and non-fasted); also 900 mg/kg body weight for non-fasted males and females . No vehicle control or control	LD50 > 900 mg/kg regardless of fasting state or sex. No mortality was observed at any dose in any sex. Non-specified toxic effects were observed as follows: non-fasted males: $\geq$ 300 mg/kg; fasted males: $\geq$ 150 mg/kg, non-fasted females: $\geq$350 mg/kg; fasted females: $\geq$ 150 mg/kg LOAEL = 150 mg/kg bw	Nakazato 1988b MRID 42256360

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Rat, SD (Crj:CD), 3 or 4 males/dose, 2 or 3 females per dose, 7 weeks old	Preliminary acute oral, WAK 3839 (nitosimine metabolite: NTN 37571) by gavage in DMSO and Lutrol at doses of 300, 1000, 1400, 1800 and 2500 mg/kg body weight (males); and 1400 and 2500 mg/kg body weight (females); no control used	LD50 > 2500 mg/kg. No mortality. Non-specified poisoning symptoms reported at all doses tested. Authors report "the poisoning symptoms were rather different from those seen in the study on NTN 33893 (imidacloprid: parent compound).	Nakazato 1990 MRID 42256361

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Rat, Sprague Dawley (Crj,CD, SPF) 5/sex/dose, 7 weeks old	LD_{50}, WAK 3839 (nitrosimine metabolite: NTN 37571) by gavage in at 980, 1560, 2500 and 4000 mg/kg body weight	LD_{50} = 3560 mg/kg, 95% CL = 2840 - 4450 mg/kg, female; LD_{50} = 1980 mg/kg, 95% CL = 1490 - 2640 mg/kg, male No mortality at 980 mg/kg, males, or up to 1560 mg/kg, females. All treated rats had toxic symptoms 25 minutes to 1 hour after exposure. Resolution of symptoms was dose-related: 3,3,7,7 days for males and 1,2,6 and 9 days for females at doses of 980, 1560, 2500 and 4000, respectively. Symptoms include: mydriasis, tremor, sedation, exophthalmos, and abnormal respirations. Some had convulsions prior to death. Nasal and ocular bleeding was seen only in males. Emaciation was seen at doses of 2500 and 4000 mg/kg. Necropsy revealed abnormal findings in the lung, stomach, small intestine, spleen and trachea for both sexes, and in the bladder and thymus for males. The gastrointestinal tract was essentially non-functional, as food was retained in the stomach and fecal excretion was suppressed. No pathological abnormalities were observed among surviving rats.	Ohta 1991 MRID 42286103
Other Species, Gavage			

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Cat, 6 male and 6 female, randomized to control and treatment groups, 4.4 - 10 kg	Advantage Spot-on formula (9.1% imidacloprid), single dose by gavage in gelatin capsules at the label-use dermal application rate in exposed cats, resulting in individual doses ranging from 9.3 to 14.0 mg imidacloprid/kg body weight; gelatin capsules plus the formulation inactive ingredients minus imidacloprid was given to controls.	Five of the six controls and 3 of 6 treated cats vomited on the day of administration, with one of the treated cats vomiting again on day 3 post-treatment. Salivation and depression was seen in control cats. No other clinical signs. No mortality. This study was not conducted according to Good Laboratory Practice regulations. No necropsies were conducted because there was no mortality.	Shmidl and Arther 1996c MRID 44179802
Dog, adults, 6 Beagle, 6 mixed breed, randomized assignment to groups,; 3 males and 3 females in control group; 2 male and 4 female in exposed group	Advantage Spot-on formula (9.1% imidacloprid), single dose by gavage in gelatin capsules at the label-use rate in exposed dogs, resulting in individual doses ranging from 10.6 to 19.9 mg imidacloprid/kg body weight; gelatin capsules plus the formulation inactive ingredients minus imidacloprid was given to controls.	A Female foxhound and a male shepherd vomited upon administration of the imidacloprid capsule. No other clinical signs were recorded. No mortality. No reductions in body weight. This study was not conducted according to Good Laboratory Practice regulations. No necropsies were conducted because there was no mortality.	Shmidl and Arther 1996a MRID 44179801
Hamster, Chinese, 5 male and 5 female per group (3 exposed groups; 1 negative control and 1 positive control group), 25 -35 g, 8-12 weeks old	*In Vivo* evaluation of clastogenic effects on bone marrow: single gavage dose of NTN 33893 techncical (94.6% a.i.) at 2000 mg/kg body weight in 0.5% Cremophor and water; sacrifice at 6, 24 and 48 hours post-exposure	No clinical signs or sypmptoms. Eating behavior was described as “normal”. Mortality in 4/34 treated animals due to NTN 33893. No increased incidence of clastogenic effects in bone marrow DNA of NTN 33893 animals relative to controls	Herbold 1989b MRID 42256344

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Hamster, Chinese, 5 male and 5 female per group; 28 - 32 g, 8 - 12 weeks old	*In Vivo* evaluation of sister chromatid exchange in bone marrow: single gavage dose of NTN 33893 (95.0% a.i.)in 0.5% Cremophor and water at 0, 500, 1000 and 2000 mg/kg body weight; sacrifice at 24 hours post-exposure.	No mortality. No impact on DNA relative to controls.	Herbold 1989d MRID 42256346
Human, male	Suicide attempt: single ingestion of approximately 100 ml of 9.7% imidacloprid formulation with <2% surfactant and trhe balance N-methyl pyrrolidone (solvent)	Drowsiness, disorientation, dizziness, oral and gastroesophageal lesions, hemorrhagic gastritis, productive cough, fever, leukocytosis and hyperglycemia. Recovery 4 days after ingestion. The reporting authors offer the opinion that the formulation ingredients, especially the N-methyl pyrrolidone caused most of the symptoms.	Wu et al 2001 MRID 45596501
Mouse, NMRI, 5 male and 5 female per group,28 - 41 g, 8-12 weeks old	*In vivo* micronucleus test, single gavage dose of NTN 33893 (95.3% a.i.) in Cremophor and deionized water at 0 and 80 mg/kg body weight; sacrifice at 24, 48 and 72 hours post-exposure	Apathy, reduced motility, and difficulty breathing for up to 6 hours after exposure; no mortality. No impact on DNA relative to controls.	Herbold 1988a MRID 42256347
Mouse, NMRI, 5 males per group	*In vivo* germ cell cytogenetic assay, single gavage dose of NTN 33893 (95.3% a.i.) in Cremophor and deionized water at 0 and 80 mg/kg body weight; sacrifice at 24, 48 and 72 hours post-exposure	No mortality reported. No chromosomal aberrations in germ cells; suggests that doses which cause clinical symptoms in mice do not cause damage to reproductive cells in males (see Herbold 1988)	Volkner 1990 MRID 42256348

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Mouse, Bor: NMRI-SPF (Han), 5 male (4 weeks old, 21 - 25 g), and 5 female (4 - 5 weeks old, 20 - 24 g) per dose group	LD_{50}, BAY NTN 33893 Technical (94.2% a.i.) by gavage in 2% Cremophor EL and demineralized water (10 ml/kg) At doses of 10, 71, 100, 120, 140, 160, 250 mg/kg body weight.	LD_{50} = 131 mg/kg body weight, Confidence interval = 111.5 -156.0, male. LD_{50} = 168 mg/kg body weight, Confidence interval = 142.3 - 200.1, female. NOAEL (clinical signs): 10 mg/kg NOAEL (mortality): 71 mg/kg bw Clinical signs: apathy, labored or transient labored breathing, decreased "motility", transient staggering gait, tansient trembling and transient spasms. No gross pathology in survivors. No effects on body weight gain in any dose group. Pale or dark spleens and livers; patchy distended lungs in animals which died.	Bomann 1989b MRID 42256324
Mouse, ICR(Crj;CD-1), 5 week old, 5/sex/dose, for fasted and non-fasted studies	NTN 37571(Title page says NTN 33893, but the report clearly states NTN 37571 and makes the statement that "the poisoning symptoms seen in this study were not different from those seen in the study on NTN 33893"; NTN 37571 is a metabolite of imidacloprid) at doses of 100, 200, 300 and 450 mg/kg body weight. Vehicle = DMSO and polyethylene glycol (10 ml/kg)	Fasted males: LD_{50} = 200 mg/kg bw (110 - 340 mg/kg bw) Non-fasted males: LD_{50} = 240 mg/kg bw (150 - 340 mg/kg bw) Fasted females: LD_{50} = 200 mg/kg bw (120 - 310 mg/kg bw) Non-fasted females: LD_{50} = approximately 300 mg/kg bw Abnormal gait and respiration, exophthalmos, tremor, convulstion and click-like vocalization noted at all dose levels.	Nakazato 1988a MRID 42256325

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Mouse, NMRI, adult male and femal, 8 weeks old, 5 male and 5 females per group	In vivo micronucleus test, WAK 3839 (metabolite, 98.9 % a.i.) in 0.5% aqueous Cremophor at 0 and 100 mg/kg body weight; Sacrifice after 24, 48 and 72 hours	No mortality. Apathy, staggering gait and difficulty breathing for up to 2 hours after dosing. External appearance, behavior and physical activity returned to normal thereafter. No treatment-related clastogenic effects on bone marrow cells	Herbold 1989f MRID 42256368
Mouse, BDF1, male, 9 weeks old	*In vivo* micronucleus test pilot study: single gavage administration of NTN 37571 (metabolite, same as WAK3839, 96.4% a.i.) 0 (vehicle control), 100, 160, 200, 300 and 400 mg/kg body weight; vehicle = DMSO and polyethylene glycol (20% v/v)	30 Hours after exposure, dose-related mortality of 20, 60 and 100% at doses of 200, 300 and 400 mg/kg, respectively. No mortality was seen among mice dosed with 100 or 160 mg/kg. No treatment-related clastogenic effects in exposed mice (second study, doses up to and including 160 mg/kg)	Usami 1988b MRID 42256369

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
INTRAPERITONEAL			
Rat, Wistar (Bor: WISW (SPF-Cpb)), male (179 g, 8 weeks old), female (178 g, 10 weeks old), 5/sex/dose	LD_{50}, NTN 33893 (technical grade imidacloprid, 94.2% a.i.) in 2% Cremophor EL and 0.9% NaCl at doses of 10, 100, 160, 170, 180, 200, 250 and 500 mg/kg body weight for males; 10, 100, 150, 180, 200, 224, and 250 mg/kg body weight for females	Male LD_{50} is between 160 and 170 mg/kg Female LD_{50} is 186 mg/kg bw, confidence interval = 162 -214 mg/kg bw, slope = 3.93. NOAEL (clinical signs): 10 mg/kg, both sexes. NOAEL (mortality): 160 mg/kg bw, males; 100 mg/kg bw, females Clinical signs included apathy, labored breathing, reduced motility, dyspnea, lacrimation tremors, spasms, twitching eyelids and piloerection. Transient impact on body weight gain in males at $\geq$ 170 mg/kg bw and in females at $\geq$ 180 mg/kg bw. No gross pathology among survivors. Gross findings on liver, lungs, spleen and GI tract among mice which died.	Krotlinger 1990 MRID 42256326
Mouse, ICR(Crj;CD-1), 5 week old, 5/sex/dose	LD_{50}, NTN 37571(Title page says NTN 33893, but the report clearly states NTN 37571) at doses of 30 or 60 mg/kg body weight. Vehicle = DMSO and polyethylene glycol 400 (5 ml/kg)	LD_{50} is between 30 and 60 mg/kg body weight for both sexes. No differences in LD_{50} values or clinical signs between sexes. Sedation, tremor and convulsion are reported for all treated mice. Authors report “no specific findings in both dead animals and survivals”.	Nakazato 1988a MRID 42256325

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Mouse, NMRI, 5 male and 5 female per group, 31 - 41 g, 8 - 12 weeks old	*In vivo* micronucleus test, WAK3839 (metabolite, 98.9% a.i), single ip injection of 0 or 50 mg/kg body weight in 0.5% aqueous Cremophor; sacrifice at 24, 48 and 72 hours	No mortality or symptoms of toxicity for up to 2 hours post-treatment. No clastogenic effects in bone marrow erythroblasts comparison with negative vehicle and positive controls.	Herbold 1989e MRID 42256366
Mouse, BDF1, 5 males/dose, 8 weeks old	Pilot study for in vivo micronucleus test, WAK3839 (metabolite, 98.9% a.i.), single ip injection at 12.3, 25, 50, 75 or 100 mg/kg body weight. Vehicle = DMSO and olive oil (10% v/v)	30 hours after injection: no mortality at doses up to and including 75 mg/kg. 40% mortality (2/5) at 100 mg/kg.	
DERMAL TOXICITY			
Rat, Wistar (Bor: WSIW SPF-Cpb), 5 male (207 - 234 g), and 5 female (204 - 214 g)	LD_{50}, NTN 33893 technical imidacloprid (94.2% a.i.), single 5000 mg/kg body weight dose applied as paste in sterile 0.9% saline; occluded exposure for 24 hours; treated skin cleaned with soap and water post-exposure	LD_{50} > 5000 mg/kg body weight, males and females No mortality. No clinical signs. No treatment-related body weight reductions. No gross pathology	Krotlinger 1989 MRID 42055332
Rat, Sprague-Dawley, (Sas: CD (SD) BR), 5 male (approx. 8 weeks old), 5 female (approx. 10 weeks old)	Single dose of 2000 mg/kg (dermal limit dose) of NTN 33893 75 WP-WS (76.1% a.i.)moistened with tap water to clipped skin, occluded 24-hour exposure. Estimated dose: 25.9 - 32.5 mg/cm^2	No mortality. Urine stain in one male and 1 female was the only clinical sign. The female developed alopecia on day 5. The alopecia persisted to the end of the study. No effects on body weight gain	Sheets and Gilmore 1991 MRID 42256314

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Rat, Sprague-Dawley (Sas(CD(SD)BR), 6 male (234-271 g) and 6 female (206 - 244 g) per dose, 8-10 weeks old	Single dose of 0 or 2000mg/kg body weight of Imidacloprid BAY T-7391 10% Pour On (9.88 - 10.01% a.i.) to shaved skin, occluded 24-hour exposure	No treatment-related mortality, changes in body weigh/food consumption, clinical signs or gross lesions. NOAEL > 2000 mg formulation/kg body weight; LD_{50} > 2000 mg formulation/kg body weight	Warren 1995b MRID 43679602
Rat, Sprague-Dawley, 5 male and 5 female, 167 - 245 g, 8-9 weeks old	Acute dermal toxicity limit test. 2000 mg/kg body weight of Permatek IM 30 (31 g a.i./L) to shaved skin, occluded 24-hour exposure	No treatment-related mortality, clinical signs or findings at gross necropsy. Satisfactory body weight gain. LD_{50} >2000 mg/kg.	Pritchard and Donald 2004b MRID 46290904
Rabbit, New Zealand White, 5 male, 5 female	Single dose of 2000 mg/kg (dermal limit dose) BAY NTN 33893 2.5% Granular (2.6% a.i.) moistened with water to 240 cm^2 clipped skin (dose equivalent to 20 to 22 mg formulation/cm^2); occluded 24 hours	No deaths. No clinical signs. All animals gained body weight. No gross lesions observed at necropsy. NOAEL > 2000 mg formulation/kg body weight; LD_{50} > 2000 mg formulation/kg body weight	Sheets 1990b MRID 4205325
Rabbit, New Zealand White, 5 male, 5 female	Single dose of 2000 mg/kg (dermal limit dose) BAY NTN 33893 240 F.S.to 240 cm^2 clipped skin ; occluded 24 hours. Estimated dose: 20.5 - 24.0 mg/cm^2.	No mortality. Clinical signs = erythema at the dose site of 2 females; muscle fasciculations in 1male and 1 female. Clinical signs resolved by day 2. No gross lesions	Sheets 1990g MRID 42056315
DERMAL IRRITATION			
Rabbit, New Zealand White, 3 male, 3 female	4-hour occluded application of BAY NTN 33893 2.5% Granular (2.6% a.i.) to shaved skin	No signs of erythema or edema at dose site 30 minutes, 60 minutes, or 24, 48 or 72 hours after patch removal. No signs of irritation. Primary irritation index = 0.00. **Not a primary dermal irritant.**	Sheets 1990d MRID 42055328

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Rabbit, White (HC:NZW), 3 male	4-hour occluded application of 500 mg BAY NTN 33893 Technical (94.2% a.i.) applied as paste made with water to shaved skin.	No edema or irritation up to 7 days post-exposure. **Not a skin irritant.**	Pauluhn 1988c MRID 42055335
Rabbit, New Zealand White, 6 male, adult	4-hour occluded application of 500 mg BAY NTN 33893 75 WP-WS (76.1% a.i.) applied as paste made with water to shaved skin.	Erythema (Grade 2) at dose site in 5/6 and edema (Grade 1) in 1/6, 1 hour after application. All irritation gone by day 7. **BAY NTN 33893 75 WP-WS is minimally irritating to skin.**	Sheets and Phillips 1991c MRID 42256320
Rabbit, New Zealand White, 3 male, 3 female, adult	4-hour occluded application of 500 mg BAY NTN 33893 70 WG (% a.i. not specified) applied to shaved skin.	Slight erythema in 3/6 at 4-hours, and in 2/6 at 24 hours. Slight edema in 2/6 at 4 hours. No signs of irritation at 24 hours. **BAT NTN 33893 70 WG is slightly irritating according to the criteria of Seabaugh and Vocci.**	Wakefield 1996b MRID 46234904
Rabbit, New Zealand White, 3 male, 3 female, adult	4-hour occluded application of 500 mg BAY NTN 33893 240 F.S. (23.1% a.i.)	No erythema or edema in any animal. **BAY NTN 33893 240 F.S. is not a primary dermal irritant.**	Sheets 1990i MRID 42256321
Rabbit, New Zealand White, 6 male, young adult	4-hour occluded application of 500 mg Imidacloprid (BAY T-7391) 10% Pour On (9.88 - 10.01% a.i.)	Erythema in 1/6 rabbits 24 hours after removal of patch; resolved by 48 hours. No other occurences. **Imidacloprid (BAY T-7391) 10% Pour On is a mild irritnt**	Warren 1995d MRID 43679605
Rabbit, New Zealand White, 3 male, 3 female, young adult	4-hour occluded application of 0.5 ml Pointer Insecticide (5% a.i.)	**Pointer Insecticide is a Category IV slight or mild irritant**	Robbins 1996b MRID 44137602

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Rabbit, New Zealand White, 2 male	4-hour occluded exposure to 0.5 ml Permatek IM30 (32 g a.i./L).	No erythema or edema in either rabbit at any observation point. **Not a dermal irritant.**	Pritchard and Donald 2004d MRID 46290906
DERMAL SENSITIZATION			
Guinea Pig, Hartley albino, males, 15 BAY NTN 33893 2.5% Granular test group, 5 BAY NTN 33893 2.5% Granular non-induced control, 5 DNCB positive control test, 5 DNCB noninduced control	Test groups received topical induction applications (4 cm x 4 cm occluded patch: 6-hour exposure duration) on days 0, 7 and 14: 0.4 g BAY NTN 33893 2.5% Granular moistened powder. DNCB was applied at 0.1% (w/v) in 50% (v/v) ethanol/deionized water. Single challenge application on day 27 (occluded patch: 24-hour duration). Application site wiped with water-moistened paper towel after exposure to remove all substance applied.	Positive response to DNCB in all test animals. **BAY NTN 33893 2.5% Granular does not cause dermal sensitization.** No irritation. No effects on body weight gain in positive controls or BAY NTN 33893 2.5% Granular test animals.	Sheets 1990e MRID 42055329
Guinea Pig, SPF DHPW, male (5- 8 weeks old), 10 controls (first challenge); 10 controls (second challenge); 20 test animals	BAY NTN 33893 (94.2% a.i.)formulated with Cremophor EL 2% (v/v) in physiological saline: Intradermal induction: 1%. Topical induction: 25%. Topical challenge: 3%,25%. Controls treated with Cremophor EL 2% (v/v) in physiological saline	No skin reaction in either treated animals or controls. **NTN 33893 Technical is not a skin sensitizer**.	Ohta 1988 MRID 42055336

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Guinea Pig, Hartley Albino, adult male, 5/dose	Range-Finding study: 24-hour occluded exposure to BAY NTN 33893 240 F.S. in deionized water at doses of 1, 10, 25, 50, 100 % (w/v).	No evidence of irritation at any dose	Sheets 1990j MRID 42256323
Guinea Pig, Hartley Albino, adult male, 15 BAY NTN 33893 240 F.S. test, 5 BAY NTN 33893 240 F.S. Control; 5 DNCB positive test, 5 DNCB control	Sensitization test: 6-hour Topical induction on days 0, 7 and 14 with undiluted BAY NTN 33893 240 F.S. or 0.1% DNCB in 50% ethanol/deionized water. 24-hour Topical challenge on day 27: undiluted BAY NTN 33893 240 F.S. or 0.1% DNCB Control: challenge only with BAY NTN 33893 240 F.S. or DNCB	No response among any BAY NTN 33893 240 F.S. test animals. Positive response, as expected in DNCB positive test controls. **BAY NTN 33898 240 F.S. does not cause dermal sensitization.**	

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Guinea Pig, Hartley Albino, adult male, 5/dose Guinea Pig, Hartley Albino, adult male, 15 BAY NTN 33893 WP-WS test, 5 BAY NTN 33893 WP-WS Control; 5 DNCB positive test, 5 DNCB control	Primary irritation study: 24-hour occluded exposure to BAY NTN 33893 75 WP-WS in deionized water at doses of 1, 2.5, 5, 7.5, 10, 25, 50, 100 % (w/v). Sensitization test: 6-hour Topical induction on days 0, 7 and 14 with 7.5%BAY NTN 33893 WP-WS or 0.1% DNCB in ethanol/deionized water. 24-hour Topical challenge on day 27: 7.5% BAY NTN 33893 WP-WS or DNCB Control: challenge only with 7.5% BAY NTN 33893 WP-WS or DNCB	Grade 1 erythema, red zones, or crusts at dose site in animals dosed with ≥10% BAY NTN 33893 WP-WS. No response among any BAY NTN 33893 WP-WS test animals. Positive response, as expected in DNCB positive test controls**. BAY NTN 33898 WP-WS does not cause dermal sensitization.**	Sheets and Phillips 1991d MRID 42256322
Guinea Pig, Hartley Albino, Adult male, 15 induced and 15 non-induced (control)	Imidacloprid (BAY T-7391) 10% Pour On, undiluted, 0.4 ml/application. 6-hour Topical induction on days 0, 7 and 14. Topical challenge on day 28. Separate positive control study with DNCB was conducted to validate the results	No treatment related erythema, edema or clinical signs in any animal at any time (either induced or non-induced rabbits). Separate positive control study with DNCB was conducted to validate the results. Imidacloprid (BAY T-7391) 10% **Pour On is neither a dermal irritant nor a dermal sensitizer in guinea pigs.**	Warren 1995e MRID 436796006

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Mouse, CBA/Ca strain, 5 females/dose, young adult	Local lymph node assay for sensitization. 0 (vehicle) , 25%, 50% or 100% Permatek IM 30 (32 g a.i./L) applied to dorsum of each ear for 3 consecutive days, followed by intravenous injection of ^{3}H-methyl-thymidine 3 days later. Vehicle = acetone in olive oil (4:1 v/v).	No mortality or clinical signs. Body weight gain considered normal. No difference between controls and any dose with regard to stimulation of T-Cell proliferation in draining auricular lymph nodes. **Permatek IM 30 is not a sensitizer.**	Pritchard and Donald 2004e MRID 46290907
INHALATION			
Rat, Sprague-Dawley, 6 male, 6 female exposed; 6 male, 6 female sham-exposed	4-hour chamber exposure to BAY NTN 33893 2.5% Granular (2.6% a.i.), as dust, at a gravimetrically determined air concentration of 5092 mg/m^3 (17040 mg/m^3 nominal); nose-only exposure. Sham-exposed controls exposed only to air	No deaths. No clinical signs. No statistically significant changes in body weight with respect to controls. No gross lesions at necropsy. NOAEL: >5092 mg formulation/m^3 LC_{50}: >5092 mg formulation/m^3	Warren 1990a MRID 42055326 and Warren 1990c MRID 42286102 (supplemental submission)
Rat, Wistar (Bor: WSIW SPF-Cpb), 160 - 210 g, 8 - 10 wks old, 5/sex/concentration; air control; vehicle control	4 hour chamber exposure to NTN 33893 Technical as aerosol in polyethylene glycol E 400 (2 lower concentrations) and powder dust (2 highest concentrations); Analytically determined concentrations: 69, 1220, 2577 and 5323 mg NTN 33893/m^3 , with particle sizes size $\leq$ 5 um at 100, 11, 6 and 4 percent, respectively	LC_{50} > 5323 mg NTN 33893/m^3 No mortality. No signs or symptoms in controls or 69 or 1220 mg/m3 groups. Difficult breathing, reduced mobility, piloerection at 2577 and 5323 mg/m3. Slight tremors at 5323 mg/m3. All groups clinically normal 1 day post-exposure. Marginally reduced body wight gain in both sexes at highest concentration. No gross pathological findings at any level of exposure	Pauluhn 1988a MRID 42055333 Pauluhn 1988d MRID 42286101 (supplemental submission)

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Rat, Sprague-Dawley (Sas: CD(SD: BR)) 6/sex/dose, 6 8 weeks old, 186 - 244 g males, 177 - 230 g females	4-hour nose-only exposure to NTN 33893 75% WP-WS as a liquid aerosol at analytically determined concentrations of 2110, 2810 or 2990 mg/m^3; sham-exposed controls received room air	LC_{50} = 2650 mg/m^3, males LC_{50} = 2750 mg/m^3, females LOAEL = 2110 mg/m^3, both sexes Clinical signs = ataxia, convulsions, hypoactivity, moribundity, nasal stain, tremors, unthriftiness and urine stain. Recovery by day 6. Statistically significant decreases in body weight gain on day 3 in males (all doses) and females (2990 mg/m3). No gross lesions other than salivation and ventral wet stain in animals dying shortly after exposure.	Warren 1991 MRID 42256316
Rat,Sprague-Dawley (Sas: CD(SD: BR)) 6/sex/dose, 6 8 weeks old, 228 - 275 g males, 189 - 230 g females	4-hour nose-only exosure to NTN 33893 240 F.S. as a liquid aerosol at analytically determined concentrations of 5060 or 5330 mg/m^3; sham-exposed controls received room air	LC_{50} > 5330 mg/m^3 LOAEL = 5060 mg/m^3 Mortality $\leq$ 50% all test groups; Hyperactivity, dyspnea, lethargy and tremors on day of exposure at both concentrations tested. Recovery by day 2. No gross lesions. No substantial reductions in body weight gain, except in low-dose males on day 3.	Warren 1990b MRID 42256317
Rat, Sprague-Dawley (Sas: CD(SD)BR), 6 male (203-228 g) and 6 female (189 - 211 g) per dose, 7-8 weeks old	4-hour nose-only exposure to either air or 2415 mg/m^3 Imidacloprid (BAY T-7391) 10% Pour On (9.88 - 10.01% a.i.) as a respirable liquid aerosol (average MMAD and GSD = 1.62um and 1.51, respectively)	Oral staining was observed in females. No changes in body weight. No mortality. N gross lesions LD_{50}>2415 mg/m^3	Warren and Berry 1995 MRID 43679603

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
OCULAR			
Rabbit, New Zealand White, 3 male, 3 female	0.1 ml of BAY NTN 33893 2.5% Granular (2.6% a.i.)instilled in conjunctival sac of one eye per rabbit.	No corneal or iridal lesions. Grades 2 and 3 ocular discharge and conjunctival redness (Grade 1) in all rabbits one hour after dosing. No signs of irritation 14 days post-dosing. Classified originally as Category II Moderate eye irritant, but subsequently reduced to **Category III mild irritant**, due to absence of corneal or iris involvement, and resolution of irritation by day 7 post-dosing.	Sheets 1990c MRID 42055327; Astroff 1992 MRID 42674401(supplemental submission)
Rabbit, New Zealand White, 3 male, 3 female	0.1 ml of BAY NTN 33893 0.5% Granular (0.56% a.i.)instilled in conjunctival sac of one eye per rabbit.	No corneal or iridal lesions. Grade 2 and 3 ocular discharg, chemosis (Grades 2 and 3), and conjunctival redness (Grades 1 and 2) in all rabbits one hour after dosing. No signs of irritation 7 days post-dosing. **Mild eye irritant**	Sheets and Phillips 1990 MRID 42055320
Rabbit, New Zealand White, 3 male, 3 female	0.1 ml of BAY NTN 33893 0.62% Granular (0.71% a.i.) instilled in conjunctival sac of one eye per rabbit.	No corneal lesions, but transient iridal lesions (grade 1) were seen in 4 rabbits at 24 hours post-instillation (resolved by 48 hours). Conjunctival redness (grade 0 - 2), chemosis (grade 1,2 or 4), and discharge (grade 2 or 3) was observed in all animals (resolved by day 7). **Mild eye irritant.**	Astroff and Phillips 1992 MRID 42674402

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Rabbit, White (HC:NZW), 2 male, 1 female	24-hour exposure to 0.1 ml solution of BAY NTN 33893 Technical (94.2% a.i.) In conjunctival sac of one eye per rabbit. Eyes rinsed with saline 24 hr post-exposure	**Not an eye irritant**, based on type, intensity and chronology of findings. No effects on the cornea, iris or conjunctiva of any rabbit at any time following exposure (up to 7 days evaluated)	Pauluhn 1988b MRID 42055334
Rabbit, New Zealand White, 6 young adults	24-hour exposure to 0.1 ml (44 -46 mg) of BAY NTN 33893 75 WP-WS in conjunctival sac of one eye per rabbit.	No corneal or iridal lesions. Ocular discharge (Grade 2 or 3), chemosis (Grade 1 or 2) and conjunctival redness (Grade 1) were observed in all rabbits one hour after exposure. No signs of irritation in any rabbit 14 days after test**. Using FIFRA criteria, BAY NTN 33893 75 WP-WS is a minimal eye irritant.**	Sheets and Phillips 1991b MRID 42256318
Rabbit, New Zealand White, 3 male, 3 female, young adults	24-hour exposure to 0.1 ml of BAY NTN 33893 240 F.S. (23.1% a.i.) in conjunctival sac of one eye per rabbit.	No corneal or iridal lesions. Transient ocular discharge (Grade 1), redness (Grade 1) and chemosis (Grade 1) of the conjunctiva in all animals, reversed in all animals by 72 hours. **Using FIFRA criteria, BAY NTN 33893 240 F.S. is a minimal eye irritant.**	Sheets 1990h MRID 42256319
Rabbit, New Zealand White, 6 young-adult males	24-hour exposure to 0.1 ml Imidacloprid BAY T-7391 10% Pour On (9.88 - 10.01% a.i.) in conjunctival sac of one eye per rabbit.	Corneal opacity, iridal irritation, conjunctival redness, chemosis and ocular discharge in all rabbits (1-48 hours). All signs resolved by day 14. Using FIFRA criteria, Imidacloprid (BAY 7-7391) 10% Pour On is a **Moderate eye irritant**	Warren 1995c MRID 43679604

Appendix 1: Acute toxicity of imidacloprid, imidacloprid metabolites and formulations of imidacloprid to experimental mammals.

Species	Exposure	Response	Reference
Rabbit, New Zealand White, 6 young-adult males	24-hour exposure to 0.1 ml Imidacloprid Pointer Insecticide (5% a.i.) in conjunctival sac of one eye per rabbit.	Corneal involvement which resolved by day 17 in all animals tested**. Category II moderate eye irritant.**	Robbins 1996a MRID 44137601
Rabbit, New Zealand White, 2 female adult	24-hour exposure to 0.1 ml Permatek IM 30(32 g a.i./L) in conjunctival sac of one eye per rabbit	No irritation in any rabbit at any time. **Not an eye irritant**	Pritchard and Donald 2004c MRID 46290905

Appendix 2: Longer-term toxicity studies in mammals

Species	Exposure	Response	Reference
Short Term Multiple Dermal			
Rabbit, HC-NZW, 5 male (3.00 kg), 5 female(2.92 kg) per group, 13 weeks old	6-hr/day, 5 days/week, 3 week occluded application exposure to NTN 33893 Technical (95.0% a.i.) to shaved skin at 0 or 1000 mg/kg body weight. Vehicle = 2% Cremophor EL in physiological saline (1.5 ml/kg bw)	No treatment-related mortality. No effects on food consumption, body weight gain. No significant differences between controls and treated animals in clinical chemistry values, blood formation or cell counts, clinical chemistry, organ weights, histopathological findings, or gross pathology. No treatment related skin changes.	Flucke 1990 MRID 42256329
Short Term Multiple Inhalation			
Rat, Wistar (Bor: WSIW SPF-Cpb), 160 - 210 g, 8 - 10 wks old, 10/sex/concentration; air control	Range-finding study for acute toxicity study MRID42055333 reported in Appendix 1: 5 x 6- hour exposures to NTN 33893 Technical as powder dust. Nominal concentrations: 20, 100, 500 mg/m^3. Analytically determined concentrations: 20, 109, and 505 mg NTN 33893/m^3 , with particle size $\leq$ 5 um at 54, 57 and 18 percent, respectively.	No mortality. No clinical signs. No effects on liver or lung to body wieght ratios. No treatment-related histopathologic changes in liver or lung at any concentration. No liver enzyme-related hapatotoxicity (Serum-ALAT, - ASAT, GLDH). NOAEC: 20 mg/m^3 Concentration-related induction of MFOs at 109 mg/ m^3 and higher; "Transient influence on body weights" at 109 mg/m^3 and higher; Dark spleen and lower erythrocyte count at 505 mg/m^3;	Pauluhn 1988a MRID 42055333 and Pauluhn 1988d MRID 42286101 (supplemental submission)

Appendix 2: Longer-term toxicity studies in mammals

Species	Exposure	Response	Reference
Rat, Wistar (Bor: WISW (SPF-Cpb), 10/sex/dose, 160-200 g., 2-3 months old.	4 weeks, 6 hr/day, 5 days/week exposure to mean analytical concentrations of 5.5, 30.5 and 191.2 mg NTN 33893 (95.5% a.i.) dust/m^3 air under dynamic conditions, air-exposed controls. Particle constitution of dust was considered respirable to the rat; head-nose only exposure"	NOAEC: 5.5 mg/m^3 NOAEL: 2.4 mg NTN 33893/kg body weight/day $\geq$30.5 mg/m^3: induction of hepatic mixed-function oxidases. 191.2 mg/m^3: statistically significant reduction in body weight gain (males only); slight depression in heart and thymus weights, and increase in liver weight (females only); slight depression in hematocrit and low-grade reduction in plasma proteins attributed to slight hpervolemia (males); increased blood coagulation time and statistically significant elevation in pH of the urine with respect to controls were considered to result from functional hepatic changes (females)	Pauluhn 1989 MRID 42273001

Appendix 2: Longer-term toxicity studies in mammals

Species	Exposure	Response	Reference
Subchronic Dietary (15 days to 90 days)			
Dairy Cow, 3/dose	28-day study residue study. 0, 5 (1 dose), 15 (3 doses) and 50 (10 doses) mg NTN 33893(97.6% a.i.)/kg feed via bolus capsules	No effects on body weight, food consumption or milk production. No effects relative to controls on weights of muscle, fat, liver or kidney at day 28 sacrifice. Imidacloprid, and its olefin, 6-chloronicotinic acid, guanidine and hydroxy metabolites were monitored in milk and tissues. Milk: Residues were not detected in the milk of controls or in cows given 1x 5 ppm dose on days 0, 1, 13 or 28 after exposure (0.02 ppm detection limit). Residues reached a plateau of 0.04 ppm and 0.14 ppm at doses of 3 x 15 and 10 x 50 ppm directly after the first exposure. Residues decreased with time. Muscle: Residues below detection (<0.02 ppm) in 1x 5 ppm cows; 0.03 ppm in 3 x 15 ppm cows and 0.12 ppm in 10 x 50 ppm cows. Fat: Residues (0.06 ppm) detected only in 10 x 50 ppm cows Liver: residues found at 0.05, 0.13 and 0.49 ppm from lowest to highest dose cows Kidneys: residues found at 0.03, 0.1 and 0.3 ppm from lowest to highest dose cows	Heukamp 1992a MRID 42556139 Murphy 1994a MRID 43143206 (additional information)

Appendix 2: Longer-term toxicity studies in mammals

Species	Exposure	Response	Reference
Dog, Beagle, 4 male, 4 female per group, 18 - 20 weeks old, 4.9 - 8.2 kg	13-week exposure to NTN 33893 Technical (95.3 % a.i.) At 0, 200, 600 and 1800 ppm (1200 ppm from wk 4 due to low food consumption) in the diet. These concentrations correspond to measured doses of 0, 65.2, 191.2 and 342.1 mg/dog/day.	No reduction in body weight gain in treated groups, except at the 1800 ppm concentration. There was no statistically significant difference between controls and treated dogs when the highest concentration was reduced to 1200 ppm. No mortality. No effects on hematology, liver and kidney function, histopathology. Trembling, independent of feeding time was observed in all 600 and 1800 ppm dogs up to the fifth week of the study. The authors attached no toxicological significance to these findings, as these symptoms were not observed in either a comparative pilot study (cf. Pages 292 - 298 in the report Annex) at a dose of 1200 ppm or in a chronic dog study at levels up to and including 2500 ppm." Reviewer disagrees based on common findings in other studies and species. **Reviewer NOAEL: 200 ppm**	Ruf 1990 MRID 42256328

Appendix 2: Longer-term toxicity studies in mammals

Species	Exposure	Response	Reference
Dog, Beagle, 2 male (8.6 kg, 4-6 months old) and 2 female (7.9 kg 4-6 months old) per dose	28-Day range-finding study: 0, 200, 1000, and 5000 ppm NTN 33893 Technical (92.85 % a.i.) in the diet. These concentrations correspond to 0, 7.3, 31.0 and 49.0 mg/kg body weight/day.	5000 ppm: all dogs died or were sacrificed. Tremor and ataxia. Marked weight loss. Histopathological confirmation of adverse effects on liver (atrophy, pigmentation of kupfer cells, hypertrophy), pancreas (decreased zymogen content), testes (tubular degeneration), thyroid (follicular atrophy), bone marrow (atrophy), thymus (involution), an salivary glands (acinar atrophy) 1000 ppm: no clincial signs; transient reduction in food consumption; no effect on body weight gain; no treatment related pathology 200 ppm: no clinical signs or reduction in food consumption; no effect on body weight gain No treatment-related effects on eyes or hearing at any dose.	Bloch 1987 MRID 42256330
Rat, Wistar (WISW SPF-Cpb), 10 male (69 g), 10 female (69 g) per dose, 5-6 weeks old	98- Day range-finding study: 0, 120, 600, 3000 ppm NTN 33893 (92.8% a.i.) in the diet.	NOAEL: 120 ppm >600 ppm: reduced body weight gain 3000 ppm: increased food consumption; decreased blood glucose and cholesterol levels; liver effects (multifocal group cell necroses, elevated alkaline phosphatase); low-grade degenerative changes in testicular tubuli.	Eiben 1988a MRID 42256334

Appendix 2: Longer-term toxicity studies in mammals

Species	Exposure	Response	Reference
Rat, Wistar (WISW, SPF Cpb), 10 male (84 g), 10 female (77 g) per concentration, 5-6 weeks old	96 day exposure to NTN 33893 (technichal grade imidacloprid, 95.3% a.i.) in feed at concentrations of 0, 150, 600, 2400 ppm; recovery groups at 0 and 2400 ppm diet for 14 weeks, then 4 weeks with no exposure; measured doses for males: 0, 14.0, 60.9 or 300.2 mg/kg body weight/day; females: 0, 20.3, 83.3 or 422.2 mg/kg body weight/day	NOAEL : 150 ppm, males; 600 ppm, females. Irreversible reduction in body weight gain (retarded growth) at concentrations $\geq$ 600 ppm in males and in females at 2400 ppm. Increased food intake relative to body weight in 2400 ppm rats, both sexes, even after the recovery period. No effects on clinical signs, drinking water consumption, mortality, hematopoietic organs, blood, eyes, organs, organ weights, histopathology, cholinesterase activity in plasma, erythrocytes or brain, at any concentration, except for the following: liver toxicity (icreased incidence of cell necrosis, round cell infiltrates, swollen cell nuclei and cytoplasmic changes in liver and slightly raised AST and ALT) in 400 ppm males. Reduced platelet count and blood clotting (thromboplastin times) in both sexes at 2400 ppm.	Eiben 1989 MRID 42256327

Appendix 2: Longer-term toxicity studies in mammals

Species	Exposure	Response	Reference
Rat, Fischer, 18/sex/group, 12/group evaluated for neurobehavioriral characteristics, 6/group evaluated for neuropathology	13-week dietary neurotoxicity screening study, analytically determined concentrations of 0, 140, 963 and 3027 ppm technical grade Imidacloprid (97.6 - 98.8% a.i.) in the diet, corresopnding to doses of: males: 0, 9.3, 63.3 and 196 mg/kg body weight/day females: 0, 10.5, 69.1 and 213 mg/kg body weight/day	No mortality. No treatment-related clinical signs. NOAEL (body wt., food consumption): 140 ppm NOAEL (neurobehavioral functional observational battery): 963 ppm mg/kg bw/day (males); 3027 ppm mg/kg bw/day (females) NOAEL (motor/locomotor activity): 3027 ppm NOAEL (clinical chemistry): 140 ppm No treatment-related gross lesions. No microscopic lesions in skeletal muscle or neural tissues.	Sheets and Hamilton 1994 MRID 43286401
Rat, Wistar (Bor: WISW (SpF -Cpb), 15/sex/dose, approximately 5 weeks old, 82 gram males, 78 gram females	12-week exposure to WAK 3839 (nitosoimine metabolite of imidacloprid) in drinking water at concentrations of 0 (tap water), 100, 300 and 1000 ppm, measured concentrations were 0, 112, 339 and 1105 ppm. Note: test substance was administered in water because of the explosiveness of the active ingredient. 1000 ppm is near saturation.	NOAEL: 110 ppm (13 mg/kg body weight/day) $\geq$300 ppm: higher lymphocyte counts and lower numbers of polymorphonuclear cells in both sexes regarded as treatment-related. $\geq$1000 ppm: reduced sodium levels in both sexes viewed as treatment-related effect on sodium balance. Lower water consumption (approximately 16% less) than controls. No thyroid effects were noted.	Krotlinger 1992 MRID 42256362

Chronic Dietary (>90 days)

Appendix 2: Longer-term toxicity studies in mammals

Species	Exposure	Response	Reference
Dog, Beagle, 4 male (6.6 - 9.2 kg) and 4 female (5.3-7.4 kg) per dose, 4-6 months old	52-Week feeding study: NTN 33893 (94.9% a.i.) in the diet at 0, 200, 500 and 1250/2500 ppm. The concentration in the last dose group was increased from week 17 onward. Dietary concentrations correspond to average doses of 0, 6.1, 15 and 41/72 mg NTN 33893/kg body weight/day	NOAEC: 500 ppm diet NOAEL: 15 mg/kg bw/day 1250/2500 ppm: slight but statistically signficant elevated plasma cholesterol (females) and elevated liver cytochrome p450 (both sexes) with respect to controls. Slight but not statistically significant elevation in liver weight (both sexes) was considered treatment related.	Allen et al. 1989 MRID 42273002
Mouse, B6C3F, 10 male (19g) and 10 female (17 g) per dose, 5-6 weeks old	107-Day range-finding carcinogenicity study. 0, 120, 600 or 3000 ppm NTN33893 (92.8% a.i.) in the diet.	NOAEL: 120 ppm, male; 600 ppm female 600 ppm: decreased body weight gain in males; 3000 ppm: decreased body weight gain in males and females; increased food consumption per kg body weight (11% males; 41% females); functional and morphological liver changes; significantly lower absolute and relative heart weights; increased frequency of death during blood withdrawal (7/10 M; 7/10 F, compared with 0/10/sex controls.).	Eiben 1988b MRID 42256337

Appendix 2: Longer-term toxicity studies in mammals

Species	Exposure	Response	Reference
Mouse, B6C3F1, 50 male (20 g) and 50 female (15 g) per dose, approximately 5 weeks old	24-Month carcinogenicity study: 0, 100, 330 and 1000 ppm NTN 33893 (95.0% a.i.) in the diet; corresponds to doses: 0, 20.2, 65.6, and 208.2 mg/kg body weight/day (males); and 0, 30.3, 103.6, and 274.4 mg/kg body weight/day (females)	NOAEL: 330 ppm 1000 ppm: reduced body weight gain (up to 10% and 5% lower for males and females, respectively. Slightly lower food and water consumption in females. No effects on incidence or timing of tumors. No effects on mortality, clinical chemistry, urinalysis, hematology, organ weights. No adverse treatment-related histopathological findings.	Watta-Gebert 1991a MRID 42256335
Mouse, B6C3F1, 50 male (25 g) and 50 female (21 g) per dose, approximately 7-8 weeks old; 10 additional mice per sex and dose were included for interim sacrifice	Supplementary 24-month carcinogenicity study: 0 and 2000 ppm NTN 33893 (95.0% a.i.) in the diet. Equivalent to doses of 413.5 (males) and 423.9 (females) mg imidacloprid/kg body weight/day	No treatment-related effects on the incidence or timing of tumors. 2000 ppm: Adverse effects on the brain (increased incidence of mineralization of the thalamus); reduced blood cholesterol levels; statistically significant reduced mean body weight (up to 29% in males and 26% in females, with respect to controls). A "squeaking and twittering type of vocalization" was heard among the treated but not control mice from the inception of the study and throughout. No statistically significant difference in mortality between treated and control mice, but treated male mice died more frequently during manipulation (ether anesthesia for blood withdrawal, during tattooing or getting caught in automatic feeders) than did controls.	Watta-Gebert 1991b MRID 42256336

Appendix 2: Longer-term toxicity studies in mammals

Species	Exposure	Response	Reference
Rat, Wistar (Bor: WESW (SPF Cpb)), 50 male (81 g) and 50 female (76 g) per dose; 4 - 6 weeks old	24-month chronic toxicity and carcinogenicity study. NTN 33893 (95.3% a.i.) at 0, 100, 300 and 900 ppm diet; corresponds to doses of 0, 5.7, 16.9 and 51.3 mg/kg body weight/day (males); 0, 7.6, 24.9 and 73.0 mg/kg body weight/day (females).	This study is the basis for EPA's RfD of 0.057 mg/kg/day NOAEL (males): 100 ppm (thyroid) NOAEL (females): 300 ppm (thyroid) Treatment-related increased incidence of mineralization of the colloid of the thyroid follicles in males (300 and 900 ppm) and females (900 ppm). Treatment-related reductions in body weight gain were observed in both sexes at 900 ppm. No other treatment-related effects on mortality, clinical signs, clinical chemistry, opthamology, organ weights, tumor incidence or pathology. No effects on plasma, red cell or brain cholinesterase.	Eiben and Kaliner 1991 MRID 42256331
Rat, Wistar (Bor: WESW (SPF Cpb)), 50 male (90 g) and 50 female (84 g) per dose; 5 - 6 weeks old: an additional 10 rats/sex/dose were treated and sacrificed after 12 weeks for interim examination.	24-month supplementary chronic toxicity and carcinogenicity study. NTN 33893 (95.3% a.i.) at 0 or 1800 ppm. Corresponds to doses of 102.6 mg/kg body weight/day (males); and 143.7 mg/kg body weight/day (females)	Confirms adverse effect on thyroid. Statistically significant (compared with controls) treatment-related increased incidence of mineralization in the colloid of the thyroid follicles; fewer colloid aggregation sites; parafollicular hyperplasia sites with minimal intensity. Also, retardation of growth (up to 12% reduction in body weight gain). No other treatment-related effects.	Eiben 1991 MRID 42256332

Appendix 2: Longer-term toxicity studies in mammals

Species	Exposure	Response	Reference
Teratology Studies			

Appendix 2: Longer-term toxicity studies in mammals

Species	Exposure	Response	Reference
Rabbit, Chinchilla (CHbb: CH hybrid: SPF quality), 16 females per dose, 4-6 months old, 2650 - 4064 g.	NTN 33893 Technical (94.2% a.i.) at 0 (vehicle control), 8, 24 and 72 mg/kg body weight/day, days 6 through 18 post coitum, by gavage in 0.5% Cremophor EL and distilled water. Sacrifice on day 28.	Maternal: NOAEL = 8 mg/kg/day. Statistically significant dose-related reduction in food consumption during treatment at 24 and 72 mg/kg/day. Reduction in body weight gain at 24 mg/kg/day (slight, during dosing period) and 72 mg/kg/day (significant on days 11-23 and 25-26 post coitum); Reproductive: NOAEL = 24 mg/kg/day. At 72 mg/kg/day: 1 female aborted on Day 26 and 2 females had total litter resoprtions at day 28 necropsy. This post-implantation loss results in a statistically significant reduction in the number of live fetuses per dam (32.5% in comparison with control value of 4.2%). There was also a slight but statistically significant reduction in live fetuses per dam, when only dams with live fetuses at termination were considered (10.8% versus control value of 4.2%). Fetal: NOAEL = 24 mg/kg/day. Slight and not statistically significant reduction in body weight with respect to controls at 72 mg/kg/day. Also at 72 mg/kg/day, increased frequency of skeletal abnormalities and statistically significantly	Becker and Biedermann 1992 MRID 42256339

Appendix 2: Longer-term toxicity studies in mammals

Species	Exposure	Response	Reference
Rat, Wistar/HAN, 25 mated females per dose, 11 weeks old, 184-240 g.	NTN 33893 Technical (94.2% a.i.) at 0 (vehicle control), 10, 30 and 100 mg/kg body weight/day, days 6 through 15 post coitum, by gavage in 0.5% Cremophor EL and distilled water. Sacrifice on day 21	Maternal: NOAEL= 10 mg/kg/day. Statistically significant reduction in food consumption at all doses; reductions in body weight gain at 30 (marginal) and 100 (significantly) mg/kg/day Reproductive: NOAEL = 100 mg/kg/day. No statistically significant treatment-related effects at any dose for any variables assessed: mean number of implants, fetuses, resorptions. Fetal: NOAEL = 30 mg/kg/day. Slightly increased incidence of wavy ribs at 100 mg/kg/day (7/149 fetuses; 5/25 litters) in comparison with vehicle controls (2/159 fetuses; 1/25 litters). No other treatment-related effects.	Becker et al. 1992 MRID 42256338

Appendix 2: Longer-term toxicity studies in mammals

Species	Exposure	Response	Reference
Rat, Wistar (Crl:W(HAN) BR, nonpregnant and nulliparous on arrival, 12 weeks of age paired with males to yield 21 mated females, approximately 25/dose; 20 litters per dose formed from litters with at least 8 pups and 3 male and 3 females, were culled to 8 pups (as closely as possible to 4 male and 4 female)	Developmental Neurotoxicity Screening Study. Technical-grade imidacloprid (98.2 - 98.4% a.i.) administered from gestation day 0 through lactation day 21 at dietary concentrations of 0, 100, 250 and 750 ppm (measured concentrations: 0, 95.5, 227 and 691 ppm); resultant doses for females during gestation: 0, 8.0 - 8.3, 19.4 - 19.7, and 54.7 - 58.4 mg/kg bw/day; during lactation: 0, 12.8 - 19.5, 30.0 - 45.4, and 80.4 - 155.0 mg/kg bw/day.	No effects on reproduction variables including the fertility index or gestation length. Maternal: 14% reduction in food consumption at highest dose. No effect on body weight, no clincal signs**.** **NOAEL: 250 ppm**. F1 Offspring: Decreased body weight gain and reduced activity in the figure-eight maze relative to controls at 750 ppm on post-natal-day (PND) 17(both sexes) and PND 21(females only). No other compound-related effects (acoustic startle habituation, passive avoidance, water maze, ophthomology, gross lesions, brain weight, brain morphometry or microscopic pathology of the brain, neural tissues or skeletal muscle). The only adverse effect persisting to termination of study was a 4% deficit in body weight, relative to controls, among high-dose males. **NOAEL: 250 ppm**	Sheets 2001 MRID 45537501

Appendix 2: Longer-term toxicity studies in mammals

Species	Exposure	Response	Reference
Multigeneration Reproduction Studies			
Rat, Wistar/HAN, 30 male (123 - 169 g) and 30 female(81 - 137 g) per dose, 5-6 weeks old at start of exposure for parental generation; breeding at approximately 17 weeks old	NTN 33893 Technical (94.4 - 95.4% a.i.) at 0, 100, 250 and 700 ppm in the diet; Parental exposure for 84 days pre-mating; during mating, gestation, and lactation, and during breeding of the F1A and F1B litters. At noon after day 21 post-partum, 26 male and 26 female pups per group were selected to form the F1 parents. F1 exposure was considered to begin when rats were 7-8 weeks old. Exposure continued throughout growth (108 days pre-mating) and during pairing, gestation and lactation periods for breeding the F2A and F2B litters.	NOAEL = 250 ppm (20 mg/kg body weight/day) for reproductive effects 700 ppm: reduced food consumption in P and F1 generations, both sexes. Reduced body weight gain in first part of the treatment of P generation.; lower mean body weight in F1 throughout the study; reduced mean body weight and body weight gain in pups of all generations (F1A, F1B, F2A, F2B) throughout the study. No teratogenic effects were observed.	Suter et al. 1990 MRID 42256340

Appendix 3: Toxicity of imidacloprid and imidacloprid formulations to birds

Species	Exposure	Effects	Reference
Single Dose Gavage			
Bobwhite quail (*Colinus virginianus*), 20-week old, 5 male, 5 female per dose group	technical grade imidacloprid (97.4% a.i.) at 0, 25, 50, 100, 200, 400 or 800 mg/kg body weight	LD_{50} = 152 mg/kg body weight, 95% CI = 103 - 227 mg/kg bw, NOAEL (mortality, clinical signs) = 25 mg/kg body weight Clinical signs: fluffed feathers, ataxia hypo-reactivity, immobility and wing drop. Significantly reduced body weight on post-exposure day 7at doses $\geq$ 100 mg/kg bw, with significantly decreased food consumption at 800 mg/kg bw.	Toll 1990a MRID 42055308
Canary (*Serinus canarius*), 5 per dose	Acute LD50 study with technical grade NTN 33893 (94.8%) in Cremophor EL/water at doses of 10, 12.5, 25 and 50 mg/kg body weight	NOAEL (mortality) = 12.5 mg/kg bw LOAEL (clinical signs) = 10 mg/kg bw, clinical signs including apathy and “cramps” and “jerks” (reviewer’s amateur translation from German to English) LD_{50} = 25-50 mg/kg bw Mortality in 1/5 and 5/5 at 25 and 50 mg/kg bw, respectively.	Grau 1994b MRID 43310403

Appendix 3: Toxicity of imidacloprid and imidacloprid formulations to birds

Species	Exposure	Effects	Reference
House Sparrow (*Passer domesticus*), adult, wild-capture, 7 per dose group	NTN 33893 2.5 Granular (2.5% a.i.) at 0, 1.5, 3, 6, 12, 25 and 50 mg a.i./kg body weight	LD_{50} = 41 mg a..i./kg body weight (419 granules per sparrow), 95% CI = 24 - 260 mg/kg bw, NOAEL (clinical signs) = 3 mg a.i./kg body weight Mortality at doses $\geq$ 12 mg a.i./kg bw Clinical signs: ataxia, hypo-reactivity, loss of flight, diarrhea, immobility and moribundity on day of administration: surviving birds fully recovered. No statistically significant effect on body weight, though weights of dead birds were not included in the analysis. Evaluation of feed consumption was not possible due to complications.	Stafford 1991 MRID 42055309

Appendix 3: Toxicity of imidacloprid and imidacloprid formulations to birds

Species	Exposure	Effects	Reference
Japanese Quail, (*Coturnix cot. japonica*), 5 male and 5 female per dose, 9-12 weeks old	Acute oral study with technical grade NTN 33893 (95.3%) at concentrations of 0, 2.5, 5, 10, 20, 40, and 80 mg/kg body weight	LD_{50} = 31 mg a..i./kg body weight, 95% CI = 22 - 50 mg/kg bw, NOAEL (mortality) = 5 mg/kg bw; all deaths occurred in first 24 hours NOAEL (toxic signs) = 3.1 mg a.i./kg body weight. (measured concentration for 2.5 mg/kg bw nominal concentration). Clinical signs, ranging from slight apathy, tumbling and ptosis at 5 mg/kg bw to unconsciousness at 80 mg/kg bw, were reversible in surviving birds. Food consumption and weight gains were comparable to controls, except for the sole surviving bird in the 80 mg/kg bw group: food consumption was almost zero during the treatment period, but returned to almost normal during post-treatment, with no effect on weight gain.	Grau 1988b MRID 43310401
Pigeon (columba livia), 5 males and 5 females per dose	Acute oral study with NTN 33893 (98.4% a.i.) via talc carrier in gelatine capsules at doses of 12.5, 25, 50 and 100 mg/kg body weight	NOAEL (mortality): 12.5 mg/kg bw LOAEL (clinical signs): 12.5 mg/kg bw, clinical signs including apathy, cramps and prone position. LD_{50}: 25 mg/kg bw (female); 25 - 50 mg/kg bw (male).	Grau 1994b MRID 43310404

Acute Dietary

Appendix 3: Toxicity of imidacloprid and imidacloprid formulations to birds

Species	Exposure	Effects	Reference
Bobwhite Quail (*Colinus virginianus*), 10-day old, 10 per concentration; 2 groups of 10 unexposed controls	5-day dietary exposure to technical grade NTN 33893 (94.8% a.i.) at nominal dietary concentrations of 78, 156, 312, 625, 1250, 2500 and 5000 ppm , corresponding to mean measured concentrations of 69, 145, 285, 567, 1168, 2290 and 4649 ppm a.i.	LC_{50} = 1420 ppm, 95% CI = 713 - 4503 ppm; LOAEC (mortality) = 69 ppm. Mortality observed $\geq$ 69 ppm; Clinical signs among dying birds include: wing drop, ataxia, hypo-reactivity, immobility and diarrhea. Significantly decreased body weight on day 5 at concentrations $\geq$ 567 ppm; However, exposed birds gained weight equal to controls during the post-exposure observation period (days 5 - 13). Significantly decreased food consumption $\geq$ 285 ppm during exposure period only (food aversion), with birds $\geq$ 2290 ppm only continuing to have decreased consumption during the observation period.	Toll 1990b MRID 42055310
Mallard Duck (*Anas platyrhynchos*), 10-day old, 10 per concentration; 2 groups of 10 unexposed controls	5-day dietary exposure to technical grade NTN 33893 (94.8% a.i.) at nominal dietary concentrations of 78, 156, 312.5, 625, 1250, 2500 and 5000 ppm , corresponding to mean measured concentrations of 69, 150, 270, 622, 1228, 2474 and 4797 ppm a.i.	LC_{50} > 5000 ppm. No mortality. Signs of ataxia in 1/10 at 2474 ppm. No treatment-related lesions upon post-mortem examination. Significantly decreased body weight on day 5 at $\geq$150 ppm. Food consumption trends support the observed decrease in body weight and the hypothesis that imidacloprid-treated food was not palatable.	Toll 1991a MRID 42055311

Appendix 3: Toxicity of imidacloprid and imidacloprid formulations to birds

Species	Exposure	Effects	Reference
Japanese Quail, (*Coturnix cot. japonica*), 10 per concentration, 10 days old	5-day dietary exposure to technical grade NTN 33893 (97.7% a.i.) at nominal dietary concentrations of 0, 313, 625, 1250, 2500 and 5000 ppm diet.	Preliminary report: 1/10 mortality at 313 ppm. 100% mortality at remaining test concentrations. No control birds died. Clinical signs included apathy, diarrhea and narcotic effects. The survivors at the lowest test concentrations were symptom free by day 6.	Grau 1994a MRID 43310402
focal species: American Robin (Turdus migratorius), northern cardinal (Cardinalis cardinalis), gray catbird (Dumetella carolininensis), blue jay (Cyanocitta cristata), brown thrasher (Toxostoma rufum), northern mockingbird (Mimus polyglottis), rufus-sided towhee (Pipilo erythropthalmus), 8 golf courses, 1 treatment and 1 control plot each	Acute toxicity field study with Merit 0.62% Granular applied to golf course turf at 0.5 lb a.i./acre (maximum proposed rate)	Average number of birds banded = 107 (control) and 98 (treated plots). All courses were similar in species diversity. The percentage of marked birds surviving 5-7 days after treatment was determined visually and by radio telemetry. There was no treatment-related effect on survival or percent mortality based on two null hypotheses (survival of focal species on treated sites is reduced by 20% or more; no difference in mortality between control and treated sites). Of the 55 intact carcasses collected after the study, only 4 had detectable residues of imidacloprid, ranging from <1% to 10% of the lowest LD_{50} for terrestrial vertebrates. Measured maximum daily mean imidacloprid residues were: 0.38 ppm in soil, 13.36 ppm in turf verdure, 0.94 ppm in puddle water and 2.21 ppm in invertebrates . Half-life of residues in soil and turf verdure were 33 and 9 days, respectively.	Toll and Fischer 1993 MRID 42737101

Appendix 3: Toxicity of imidacloprid and imidacloprid formulations to birds

Species	Exposure	Effects	Reference
Red-Winged Blackbird (Agelaius phoeniceus), wild-captured males, 8 per concentration in cup tests; 10 per flight pen in each replicate of the flight pen tests.	Cage and flight pen evaluation of avian repellency and hazard associated with imidacloprid-treated rice seed. 0, 278, 833 and 2500 ppm	**4-day two-cup tests**: birds are presented with feed in two cups: 1) control and treated seed undyed; 2) control and treated seed both dyed; 3) Control seed undyed, treated seed dyed. *Test 1* and 2) Significantly lower consumption of treated rice compared with controls in birds given choice between untreated rice and rice treated at 833 and 2500 ppm. *Test 3*) Significant reduction in consumption of treated rice versus untreated rice at all levels. Dose related increase in consumption disparity between treated and untreated cups. **4-day one-cup test**: Rice consumption measured in 4-day pre-treatment period and compared with that in 4-day treatment period. Birds given one cup at the specified treatment level, with all seed dyed. Average reduced consumption of 1.08 g/bird and 2.49 g/bird at 833 and 2500 ppm , respectively, in comparison with pre-treatment consumption levels. No difference between pre-treatment and treatment consumption rates seen at 0 or 278 ppm. **6 replicate Flight Pen tests**: 8 plots per pen, only 2 randomly selected plots were used in a test, one treated (800 grams of 2500 ppm imidacloprid-treated rice, one untreated control (800 grams untreated rice). Over a 4-day	Avery et al. 1993a,b MRID 42856201

Species	Exposure	Effects	Reference
Supplemental information for Avery et al. 1993a,b: In the flight pen studies, investigators observed an inverse relationship between the number of treated seeds removed and the mean minimum temperature during the test. Treated seed removal also appeared to be increased by the presence of predators outside the pen during trials. Residue analysis indicated that birds ingested 13-16% of the imidacloprid present on the seed. With this information, the investigators stated that birds feeding at an average rate of 6 seeds/minute (seed treated with 2500 ppm imidacloprid) would consume only a fraction of the LD_{50} dose (they used the house sparrow LD_{50} of 41 mg/kg from Mullins 1993 as the basis for comparison)			
Ringed turtle dove (*Streptopelia risoria*) and House Sparrow (*Passer domesticus*), two trials, two replicates per trial, one control and two treatment groups per replicate, with 8 birds of each species per concentration and replicate	Seed avoidance study (two trials) with imidacloprid-treated wheat and sorghum seed. Nominal (measured) concentrations on wheat: 313 (228) and 1250 (1058) ppm a.i.; on sorghum: 2500 (2354) and 5000 (4612) ppm a.i. Comparison with untreated seed for controls. 5-day pre-treatment period, followed by 2-day break, then 5-day treatment period.	Seed consumption, body weight, clinical appearance (via video camera) and survival were monitored. **Doves**: significantly reduced body weight and seed consumption in comparison with controls in both seed trials at all imidacloprid concentrations tested. Dose-related clinical signs (hypoactivity, fluffed feathers, vomiting) in all but one bird. Mortality only in trial with sorghum, with one death at 2354 ppm a.i.and 4 at 4612 ppm a.i. **Sparrows**: No treatment-related mortality. Significantly reduced body weight in comparison with controls only at 4612 ppm a.i. in the sorghum trial. Significantly reduced food consumption for all birds exposed to imidacloprid-treated seeds in comparison with controls. Clinical signs (hypoactivity, ataxia, fluffed feathers) in 2 birds at each of the imidacloprid-treated groups for the sorghum trial only.	Hancock 1994a MRID 43197501

Species	Exposure	Effects	Reference

Supplemental information for Hancock 1994a:
The investigator observed that both species learned to avoid imidacloprid-treated seed through post-digestive distress. Hancock hypothesizes that doves were more sensitive than sparrows due to differences in eating habits. Doves consumed large numbers of seed during the initial visit to the feeder, while sparrows consumed fewer seeds per visit. As such, doves were exposed to higher internal doses of imidacloprid than sparrows. Due to the slower rate of ingestion, sparrows learned avoidance, which resulted in lower exposure and toxicity. Hancock estimated the dose for doves exposed to 4612 ppm-treated sorghum to be 47 mg/kg body weight (based on observed seed consumption and regurgitation, and assumes 100% absorption of non-regurgitated seed, 38% absorption of regurgitated seed and a 150 g body weight).

Appendix 3: Toxicity of imidacloprid and imidacloprid formulations to birds

Species	Exposure	Effects	Reference
Reproduction Studies			

Appendix 3: Toxicity of imidacloprid and imidacloprid formulations to birds

Species	Exposure	Effects	Reference
Bobwhite Quail (*Colinus virginianus*), 18 pens per concentration tested, with 1male and 1 female per pen	One-generation study, 20-week dietary exposure to 0, 30, 60, 120 and 240 ppm technical grade NTN 33893 (94.8% a.i.), equivalent to mean measured concentrations fo 0, 36, 61, 126 and 243 ppm.	Parental generation: Significantly reduced body weight, but not feed consumption among males exposed to 243 ppm. No signs of toxicity, no treatment-related gross lesions at sacrifice. Two deaths (a male at 61 ppm and a female at 126 ppm were not considered compound-related). No other mortality. Reproductive variables: Significant reduction in hatchling body weights in comparison with controls at all concentrations. However, significantly increased 14-day survivor weights at 126 and 243 ppm, in comparison with controls, and equal or greater than numbers surviving among imidacloprid-treated offspring. A small decrease in eggshell thickness at 61 (0.34 mm), 126 (0.34 mm) and 243 ppm (0.33 mm), was observed in comparison with controls (0.35 mm). The difference was statistically significant for the 61 and 243 ppm birds. However, no reduction in shell strength, increase in percentage of cracked eggs or decrease in hatchability was observed at these concentrations. The investigators considered the observed effects not to be of biological significance, and state that the NOAEC for the study is 126 ppm on the basis of reduced male body weight at the higher concentration.	Toll 1991b MRID 42055312

Appendix 3: Toxicity of imidacloprid and imidacloprid formulations to birds

Species	Exposure	Effects	Reference
Mallard Duck (*Anas platyrhynchos*), 15 pens, with 1 male and 1 female per pen	One-generation study, 20-week dietary exposure to 0, 60, 120 and 240 ppm technical grade NTN 33893 (94.8% a.i.), equivalent to mean measured concentrations fo 0, 64, 125 and 234 ppm	No effects on parental birds other than sporadic significant decreases in mean weekly feed consumption. 234 ppm: Significant reduction in mean number of eggs laid per hen, resulting in reductions in mean number of hatchlings per hen, percentage of normal hatchlings of viable eggs, percentage of normal hatchlings of live three-week embryos and percentage of 14-day old survivors per hen. On this basis, the **NOAEC for the study is 125 ppm**	Toll 1991c MRID 42055313
Mallard Duck (*Anas platyrhynchos*), 15 male/female adult pairs per treatment	One-generation study, 20-week dietary exposure to 0, 60, 120 and 240 ppm technical grade NTN 33893 (95.8% a.i.), equivalent to mean measured concentrations of 0, 61, 128 and 250 ppm	**NOAEC: 128 ppm** **LOAEC: 250 ppm**. Statistically significant reduction in eggshell thickness and strength. There was a statistically significant increase in number of cracked eggs at 128 ppm but this was deemed biologically unimportant due to the lack of dose-response and lack of this finding in the previous study (Toll 1991) No clinical signs of toxicity, no effects on mortality, no treatment-related lesions and no statistically significant differences in parental body weight, food consumption, egg production, egg viability, 21-day embryo survival, hatchability, hatchling body weight, 14-day survival or survivor body weight were observed.	Stafford 1992 MRID 42480502

Appendix 3: Toxicity of imidacloprid and imidacloprid formulations to birds

Species	Exposure	Effects	Reference
Mallard Duck (*Anas platyrhynchos*), 15 adult male/female pairs per dose, one pair per cage.	Eggshell quality one-generation study. NTN 33893 (96.0% a.i.) in the diet for 19 weeks at nominal (measured) concentrations of 0, 25 (22), 40 (35) and 55 (47) ppm a.i.	No statistically significant differences in eggshell strength or thickness between controls and any treatment group. No statistically significant differences between controls and any treatment level with respect to body weight, food consumption, clinical signs (none) or mortality (none). **NOAEC: 47 ppm a.i.**	Hancock 1994b MRID 43466501

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Bees			
Honey Bee (*Apis mellifera*), 2 groups of 10 each per concentration	Acute oral and contact toxicity. Technical grade NTN 33893 (99.8% a.i.) Oral route: 0.0015, 0.0031, 0.0063, 0.0125, and 0.025 ug/bee Contact route: 0.025, 0.05, 0.10, 0.20 and 0.40ad ug/bee	48-hour oral LD_{50}: 0.0037 ug/bee, 0.0026 - 0.0053 95% CI 48-hour LOAEL: 0.0015 ug/bee 48-hour contact LD_{50}: 0.008 ug/bee, 0.0055 - 0.0119 95% CI 48-hour contact LOAEL: 0.025 ug/bee	Cole 1990 MRID 42273003

Supplemental information for Cole 1990: 48-hour oral mortalities for control, 0.0015, 0.0031, 0.0063, 0.0125 and 0.025 ug/bee are 5%, 20%, 50%, 65%, 90% and 100%, respectively. 48-hour contact mortalities for control, 0.025, 0.05, 0.10, 0.20 and 0.40 ug/bee are: 0%, 20%, 30%, 55%, 80% and 95%, respectively.

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Honey Bee (*Apis mellifera*), 2 tests, 2 replicates each application rate per test, approximately 50 bees per replicate	24-hour acute toxicity following application to alfalfa foliage, NTN 33893 240 FS, control 0.045, 0.167 and 0.5 lb a.i./acre	Mortalities assessed 2, 8 and 24 hours after caging bees with treated foliage. RT_{25} is the residual time needed to reduce chemical activity such that bee mortality is less than 25%. **Conclusion:** RT_{25} for 0.045 lb a.i./acre < 2 hours: NTN 33893 may be applied at this rate with minimal hazard to bees during early morning or late evening when bees are not actively foraging. RT_{25} for 0.167 lb a.i./acre < 8 hours: NTN 33893 may be applied at this rate with minimal hazard to bees during late evening when bees are not actively foraging RT_{25} for 0.5 lb a.i./acre = 8 hours: NTN 33893 may be applied at this rate with moderate hazard to bees during late evening when bees are not actively foraging	Hancock et al. 1992 MRID 42632901

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Honey Bee (*Apis mellifera*), seven tests conducted with bees from seven different apiaries in Germany (5), the Netherlands (1) and the United Kingdom (1). Each test used adult workers, 14-42 days old, 10 bees per dose, 3 replicates per dose	Acute oral toxicity. Technical grade imidacloprid in sucrose at nominal concentrations of 0.1 - 81 ng a.i./bee for 3-4 hours. Note: honeybees rejected sucrose solutions containing imidacloprid at concentrations of 1 mg/kg or higher, in a dose-dependent manner. This could be due either to avoidance or knockdown effect (bees immobile and thus, unable to feed)	Mortality was assessed at 4, 24 and 48 hours after dosing. Control mortalities ranged from 0 - 10% in the seven studies. Neither season nor origin of apiary impacted the measured LD_{50} values (oral or contact exposure). Oral LD_{50} = 41 to >81 ng a.i./bee Oral NOAEL (mortality) = 1.5 ng a.i/bee, with 17 - 50% mortality at higher doses ($\geq$3.1 ng/bee). Investigators noted poor fit of dose-response curve in all studies (probit analysis, non-linear regression, moving average methods all employed)	Nauen et al 2001
Bees from seven different apiaries and tests as above, 10 bees per dose, 3-5 replicates per dose	Contact toxicity. Technical grade imidacloprid sprayed on anesthetized bees at 40 to 154 ng a.i./bee	sub-lethal effects observed after 4 hours at all doses, with either death or recovery after 48 hours. 48-hour LD_{50} = 42 - 104 ng/bee. Good fitting dose-response curves.	

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Honey Bee (*Apis mellifera*),adult workers, 14-42 days old 10 bees per dose, 3 replicates per dose	48-hour oral LD_{50} test of Imidacloprid and imidacloprid metabolites	48-hour LD_{50}/ NOAEC (ng a.i./bee): imidacloprid: 41/ 1.5 olefin metabolite: <36/ 2.4 5-OH-imidacloprid: 159/ 1.2 di-OH-imidacloprid: >49/ 49 urea metabolite: >99500/ 1200 6-chloronicotinic acid: >121500/ 121500 Authors note that metabolites which contain the nitroguanidine pharmacophore (5-member ring with nitrogen-containing substituent group) are toxic to bees, whereas those without it (6-chloronicotinic acid and urea metabolite) are not.	Nauen et al 2001
Honey Bee (*Apis mellifera*), 3 cages of 20 bees each per experiment, each experiment replicated 3 times	acute oral toxicity of technical grade imidacloprid (97% a.i.) and its metabolites.	The LD_{50} values for the 4,5-dihydroxy, desnitro, 6-chloronicotinic acid, and urea metabolites were each > 1000 ng/bee (> 10,000 ug/kg). See table below for other values.	Suchail et al. 2001

LD50 values (ng/bee [ug/kg]) from Suchail et al. 2001

	Imidacloprid	5-OH-imidacloprid	Olefin
48-hour	57 [570]	258 [2580]	28[280]
72-hour	37 [370]	206 [2060]	29 [290]
96-hour	37 [370]	222 [2220]	23 [230]

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Honey Bee (*Apis mellifera*), from three apiaries in the UK (a) , The Netherlands (b) and Germany (c), 10 bees per dose	Both oral and contact LD_{50} tests conducted at 3 different facilities, separate tests of technical grade imidacloprid, and Bayer imidacloprid formulations WG70 (700 g/kg), and SC200 (200 g/L), 4-6 doses	48-hour oral/contact LD_{50} (ng a.i./bee): technical grade a: 3.7/ 81.0 technical grade b: >21/ 230.3 technical grade c: 40.9/not tested WG70 c: 11.6/ 242.6 SC 200 c: 21.2/ 59.7 NOAEL(mortality): 1.2 ng/bee Note: letter designation refers to origin of bees, as designated in the "species" column	Schmuck et al. 2001

supplemental information from Schmuck et al. 2001: LD_{50} values were converted into LC_{50} values as follows: a = [b/(20 x 1.3)] x 1000, where a = lethal concentration in mg/kg and b = oral dose in ug/bee. From the above data, this yields LC_{50} values ranging from 0.142 to 1.573 mg/kg diet, with a NOAEC of 0.046 mg/kg diet.

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Honey Bee (*Apis mellifera*) head membrane preparations	Binding studies with imidacloprid metabolites to determine displacement of ^{3}H-imidacloprid	Neither 6-chloronicotinc acid nor the urea metabolite were effective in displacing imidacloprid from its binding site even at high concentrations (0.1 mM). The affinity of the other metabolites for the imidacloprid binding site decreased in the following order: olefinic metabolite > 4-OH-imidacloprid>>4,5-OH-imidacloprid. These data support the idea that neither 6-chloronicotinic acid nor the urea metabolite are biologically active via the imidacloprid receptor in the honey bee. These results were backed up by electrophysiolotical studies with imidacloprid and its metabolites. Similar results have been reported by these investigators for aphids.	Nauen et al 2001

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Honey Bee (*Apis mellifera*), approximately 20 late summer worker bees of unknown age per treatment per replicate, three replicates	oral LD_{50} test with technical grade imidacloprid (99.8% a.i.) at 0.2 - 3.2 mg/L (2 - 32 ng/bee) yielding measured concentrations of 0, 3.2, 8.8, 32.8 and 49.5 ug/kg in sucrose solution	48-hour LD_{50} (95% confidence interval): 30.6 ng/bee (26.7 - 36.3)	Decourtye et al. 2003
	oral LD_{50} test with 5-hydroxyimidaclopri d (99.8% a.i.) at 1.25 - 20 mg/L (12.5 - 200 ng/bee), yielding measured concentrations of 0, 34.1, 83.8 and 168.4 ug/kg in sucrose solution	48-hour LD_{50} (95% confidence interval): 153.5 ng/bee (125.9 - 196.9)	

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Honey Bee (*Apis mellifera*)	Test of hypothesis that low doses of imidacloprid activate the cholinergic system, and this impacts learning. Imidacloprid in DMSO applied to thorax at 0, 1.25, 2.5, 5, 10 and 20 ng/bee	Imidacloprid at 1.25 ng/bee significantly reduced habituation of proboscis extension, and increased motor activity, independently of time. Bees receiving imidacloprid needed fewer trials to display the proboscis extension reflex (PER) and spent less time in immobility than did negative or vehicle controls (good thing). Higher doses of imidacloprid (2.5 - 20 ng/bee) showed dose-related impairment of activity relative to controls. Doses greater than 5 ng/bee had a time-dependent significant increase in gustatory threshold with respect to controls.	Lambin et al. 2001

Armengaud et al. 2000: Study of functional cytochrome oxidase (CO) histochemistry in honey bee brain. Cytochrome oxidase activity is used as a metabolic marker for neuronal activity. Chemical stimulation in the form of 50 mM potassium ion caused an increase in CO staining in the antennal lobes and to a decrease in the basal ring of calcyces. Imidacloprid injected into honeybee brains at a concentration of 10^{-4}M (~25 ng/bee) increased CO in all brain structures analyzed. However, injection of a lower dose of imidacloprid (10^{-8}M) caused a decrease in CO staining in the basal ring of calcyces and central body, while causing increases in CO in all othe structures. This suggests that the neuronal action of imidacloprid is complex, and that there may be two sub-types of nicotinic receptors sensitive to imidacloprid.

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Honey Bee (*Apis mellifera*), 4, 5, 6, 7, 8, 9 and 10 day old bees	Effects of imidacloprid exposure and age on habituation of habituation of the proboscis extension reflex (PER). Sub-lethal exposure to imidacloprid at 0.1, 1 and 10 ng/bee	Untreated bees: habituation of PER is age-dependent: older bees (7-8 days old) require significantly more trials than younger bees (4-7 day old). Imidacloprid exposure changes the habituation of PER in an anomalous way. Imidacloprid increases the number of trials for habituation in 7-day old bees at 15 minutes (all doses), 1 hour (10 ng/bee only) and 4 hours (all doses) post-exposure. Imidacloprid reduces the number of trials for habituation in 8-day old bees tested at 15 minutes and 1 hour after exposure (all doses), but increases the number of trials 4 hours post-treatment (significant difference from controls at 1 and 10 ng/bee). The dose effects and timing of the response (15 minutes, 1 hour, 4-hours) suggest the existence of two sub-types of binding receptor and the possibility that initial effects are due to imidacloprid, and later effects are due to metabolites.	Guez et al. 2001

Matsuda et al. 2001: "There is evidence for insect nAChR subtypes based on physiology, pharmacology, molecular cloning and genome sequencing studies." "Recent studies using binding assays, molecular biology and electrophysiology suggest that both alpha- and non-alpha- subunits of nAChRs contribute to interactions of these receptors with imidacloprid."

Species	Exposure	Effects [a]	Reference
Zafeiridou and Theophilidis 2004: This study supports the idea that there are two sub-types of imidacloprid binding site. The respiratory rhythm of the beetle, Tenebrio molitor was studied following exposure to low concentrations of imidacloprid. An increase in the firing of respiratory motor neurons was observed with respect to controls following treatment with 0.10 uM imidacloprid. On the other hand, treatment with 1.0 uM imidacloprid caused an abrupt increase in frequency followed by complete inhibition. The authors estimate a NOEC of 0.001 to 0.010 uM imidacloprid for effects on motor neuron firing.			
Honey Bee (*Apis mellifera*), newly emerged worker bees, 60 - 163 bees per treatment	chronic mortality (11-day exposure) in bees exposed to imidacloprid (99.4% a.i.) in sucrose: two experiments, summer and winter bees tested at concentrations ranging from 0, 1.5, 3, 6, 12, 24, and 48 ug imidacloprid/kg sucrose solution (winter) and 0,1.5, 3, 6, 12, 24 and 96 ug/kg (summer)	Statistically significant mortality, with respect to controls at 48 ug/kg in winter bees 20.5% versus 11.6% in controls) and 96 ug/kg in summer bees 17.7% versus 3.3% in controls). NOAEC (mortality) summer bees = 48 ug/kg. NOAEC (mortality) winter bees = 24 ug/kg.	Decourtye et al. 2003

Supplemental information for Decourtye et al. 2003: Assuming a sucrose density of 1227 kg/m^3 (based on experimental conditions of 500 g/L sucrose solution at 25^{o}C) and a daily solution ingestion rate of 33 ul/bee (experimentally confirmed), it is possible to convert the NOAEC to a NOAEL as follows: 24 ug/kg x 1227 kg/m3 x 10-3 m3/L x 33E-6 L/bee = 0.00097 ug/bee or 0.97 ng/bee.

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Honey Bee (*Apis mellifera*), newly emerged worker bees, 60 - 163 bees per treatment	chronic mortality (11-day exposure) in winter bees exposed to **5-OH-imidacloprid** (99.4% a.i.) at nominal concentrations of 0, 7.5, 15, 30, 60, 120, and 140 ug /kg sucrose solution	NOAEC (mortality): 120 ug/kg LOAEC (mortality); 240 ug/kg (41% mortality in comparison with 17.2% control mortality)	Decourtye et al. 2003
Honey Bee (*Apis mellifera*), 3 cages of 30 bees each per experiment, each experiment replicated 3 times	10-day chronic mortality study of imidacloprid (97% a.i.) and its metabolites (5-OH-, di-OH-, desnitro-, olefin-, and urea-imidacloprid) in 50% sucrose, each tested at concentrations of 0, 0.1, 1, and 10 ug/L (0.010, 0.1 and 1 ng compound/bee/day). Concentrations are based on the observation that bees consumed 12 ul sucrose solution per day.	control mortality did not exceed 15% in any experiment or replicate. Imidacloprid and all metabolites caused mortality within 72 hours after the onset of intoxication (trembling, tumbling, coordination problems). 50% mortality was reached by day 8 for all metabolites tested except 0.1 ug/L imidacloprid (significant lower mortality for entire duration of study in comparison with higher doses) and 0.1 ug/L 5-OH imidacloprid (reached 40% mortality by end of study). All metabolites yielded similar timing of mortality. Only imidacloprid and 5-OH-imidacloprid showed evidence of dose-response.	Suchail et al. 2001

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Honey Bee (*Apis mellifera*)	39-day feeding study to assess effect on colony development using sunflower honey dosed with imidacloprid at concentrations of 0.002, 0.005, 0.010, and 0.020 mg/kg. Concentrations were based on residue studies with sunflowers.	No adverse effects on mortality, feeding activity, wax/comb production, breeding or colony vitality were detected at any concentration, yielding an NOAEC of 0.020 mg/kg for imidacloprid. Since imidacloprid residues in pollen and nectar from sunflowers grown under field conditions are less than this value (see below) it is not likely that honeybees would adversely be affected by use of imidacloprid under field conditions.	Schmuck et al. 2001 Note: this study was conducted by Bayer AG

Schmuck et al. 2001 supplemental information: Imidacloprid residues (imidacloprid and metabolites) were not detected (detection limit = 0.0015 mg/kg) in the pollen or nectar of sunflowers grown from imidacloprid-treated seeds (dressed with Gaucho 70WS at label-recommended rate) in 3-4 different fields in two locations in Germany. Furthermore, no detectable imidacloprid residues were found in the pollen or nectar of sunflowers grown in soils which had previously hosted crops grown with imidacloprid-treated seeds. Schmuck et al. 2001 conclude: "From these findings it is evident that honeybees are not exposed to residues of imidacloprid or structurally related imidacloprid metabolites when foraging on sunflower plants, irrespective of whether these plants had been cultivated on previously imidacloprid-treated soils or had been raised from imidacloprid-dressed seed."

Laurent and Rathahao 2003: Looked at the distribution of ^{14}Cimidacloprid in sunflowers following seed treatment with Gaucho 70 WS (equivalent to 1 mg imidacloprid/seed: 30% higher than the label-recommended rate). Plants absorbed approximately 5% of the radiation on seeds, with 75% of that found in the cotyledons. Imidacloprid residues were detected in pollen at 13 $\pm$ 13 ng/g (mean $\pm$ SD, n= 5 flowers; range = below detection [0.5 ng/g] to 36 ng/g). These investigators did not assess impacts of imidacloprid on honey bees.

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Honey Bee (*Apis mellifera*), 2 cages of 50 bees each for imidacloprid treatments, 3 cages, 50 bees each for controls	chronic feeding study to assess timing of mortality at doses below the 48-hour $LD_{50.}$ Technical grade imidacloprid (99.8% a.i.) at 0, 4 and 8 ug/L in sucrose. Measured consumption was approximately 20 ul sucrose solution per bee per day. Average doses are thus, 0, 0.08 and 0.16 ng imidacloprid per bee per day.	Mortality profiles for control and imidaclorprid exposed bees differed. For imidacloprid-exposed bees, a sharp increase in cumulative mortality was observed for both doses between days 30 and 40 (>80% mortality, compared with approximately 40% mortality for controls), with 100% mortality between days 40 and 50. For controls, mortality occurred at a steady rate with time of exposure, with 100% mortality achieved on day 60. No difference in food consumption between controls and imidacloprid-exposed bees was observed.	Dechaume Moncharmont et al 2003

supplemental information for Dechaume Moncharmont et al 2003: These investigators attempt to make the point that there is an inverse relationship between severity of effect and exposure concentration. In support of this, they state that the mean survival time ($\pm$ standard error) of 28.3$\pm$5.6 days for bees exposed to 4 ug/l , is less than that of 31.3$\pm$4.1 days for bees exposed to 8 ug/l. This reviewer disagrees with the observation of inverse dose-response. Given that the means are each within the range of the other, there is likely no biologically meaningful difference between the results obtained for the different exposures.

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Bumble (Bombus impatiens), one caged colony per plot, 10 paired plots	field study in Kentucky to assess foraging on flowering white clover in turf. Paired plots, one treated one control, Merit 0.5 Granular applied at maximum label rate for white grubs (0.4483 kg a.i./ha) with irrigation; bees foraged for 30 days	**no effects** on colony vitality measured in terms of weight, number and weight of workers, number of brood chambers and honey pots, and measures of defensive response. No effects on foraging activity.	Gels et al. 2002
Bumble (Bombus impatiens), one caged colony per plot, 5 groups of three plots each	field study in Kentucky to assess foraging on flowering white clover in turf. 5 groups of 3 plots each: 2 plots sprayed with Merit 75 at a rate of 0.336 kg a.i./ha, 1 plot was irrigated with 1.5 cm water, 1 was not. One control plot. Bees foraged 28 days.	no effects on colony vitality or workers defensive response on irrigated plots. However, bees on non-irrigated plots were adversely affected with respect to bees on untreated control plots: fewer honey pots and brood chambers, fewer workers, reduced biomass of workers and lower colony weight. Queen weight was not affected. Reduced defensive response to an aggressive stimulus was also observed. Foraging activity was reduced significantly on non-irrigated plots, but not on irrigated plots, with respect to controls.	Gels et al. 2002

Supplemental information for Gels et al. 2002: A study showed that wild bumble bees did not selectively prefer or avoid plots of tall fescue and white clover treated with granular imidacloprid (Merit 0.5G applied at 0.336 kg/ha with 1.5 cm irrigation) with respect to untreated control plots.

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Honey Bee (*Apis mellifera*), newly emerged worker bees, 60 - 163 bees per treatment; summer bees versus winter bees tested	proboscis extension response (PER)as measure of reflex following 11-day feeding exposure: 99.4% pure imidacloprid (7.5 - 240 ug/kg in sucrose) and 5-hydorxyimidaclopri d (1.5 - 48 ug/kg in sucrose)	Significantly decreased response in summer bees compared with controls at 48 and 96 The NOEC for imidacloprid for proboscis reflex response among summer bees was 24 ug/kg. There was no significant difference in response between treated and untreated winter bees (NOAEC > 48 ug/kg).	Decourtye et al. 2003
	olfactory learning performance in the lab, following approximate 11-day expsoure: exposure as above	Imidacloprid significantly reduced conditioned olfactory learning responses in comparison with controls as follows: summer bees: NOEC: 6 ug/kg, LOEC: 12 ug/kg winter bees: NOEC: 24 ug/kg, LOEC: 48 ug/kg	

Supplemental information for Decourtye et al. 2003: The meaning of the above findings is unclear, given that these tests are contrived laboratory experiments which do not approximate field conditions. The authors state: "It remains to be determined whether a decrease in the olfactory learning ability as detected in the PER assay would significantly affect the foraging behaviour in such a way that bee populations would suffer severely...Further work is still needed to establish a better correlation between the behavioural responses observed under laboratory conditions and those observed in field studies."

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Honey Bee (*Apis mellifera*), colonies in outdoor flight cages	24 ug/kg technical grade imidacloprid (98% a.i.) in sucrose solution; comparison between pre-treatment periods with unmodified sucrose solution and various periods of exposure (up to 10 days) to imidacloprid-containing sucrose solution	no effect on mortality when pre-treatment and post-treatment comparison was made. Imidacloprid treatment caused a decrease in foraging activity (measured by mean sucrose consumption) when rates were compared before treatment (186±39.3 ml, n=6), during treatment (57.9±9.7 ml, n=5) , and after treatment 38.2±5.3 ml, n=5)	Decourtye et al. 2004
Honey Bee (*Apis mellifera*)	metabolism of imidacloprid. Oral exposure to 20 and 50 ug/kg bee	Regardless of dose, 70% of the administered imidacloprid was detected in bees as unchanged imidacloprid (50%), 5-hydroxy imidacloprid (9%) and olefin (8%) residues 20 minutes following exposure. Signs of toxicity but no mortality were seen at this time. Imidacloprid had a half-life of 4.5 to 5 hours, and was no longer present in bees 6 hours post-exposure. were the primary metabolites identified, peaking at 4 hours post-exposure. Mortality corresponded with appearance of olefin and 5-hydroxyimidacloprid metabolites at 4-hr post-exposure.	Suchail et al. 2004

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Beneficial predators			

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Carabid beetle (Harpalus pennsylvanicus) (preys on living or dead insects), field-captured adults	Imidacloprid label application rate for control of grubs = 0.336 kg a.i./ha		Kunkel et al. 2001
10 adult beetles per replicate; 4 replicate plots each treatment plus controls	dietary study: dog food pellets sprayed with imidacloprid at label rate and 0.5 label rate; beetles examined at 4h, 12 h, and daily for 7 days	intoxication of 100% of all imidacloprid treated beetles (both doses) between 4 hours and 1 day post-exposure; most beetles were recovered by day 7.	
10 adult beetles per replicate; 3 replicates each treatment plus controls	contact study: plots with beetles sprayed at 0.25, 0.5 and label rate; beetles examined at 4h, 12 h, and daily for 7 days	significant early intoxication (most beetles incapacitated within 4 hours, appearing dead or nearly dead; all beetles incapacitated by 1 day) followed by recovery within 4 days for more than 85% of the beetles, and by day 7 for.	
3 replicate pairs, 10 beetles each	residue study: pairs of plots sprayed at label rate: one irrigated, one not irrigated; beetles examined 48 hours after treatment	Significant residual toxicity with respect to controls was observed on non-irrigated plots only, though most of the intoxicated beetles (80%) recovered.	
control and treated beetles, 3 replicates, 15 beetles each	vulnerability to predatory ants: examination of imidaclorprid-intoxicated beetles (fed pellets treated with 0.336 kg a.i./ha) versus	Intoxicated beetles, but not untreated controls were captured by predatory ants.	

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Colpoclypeus florus (ectoparasitoid: attacks larvae of leafrollers), 5 2-4-day old adult females	48-hour acute contact toxicity. Provado 2F sprayed on insects at label rate of "48 ppm or amount/100 gal."	100% mortality when applied at 100% label application rate for apple trees	Brunner et al 2001
Colpoclypeus florus, 5 2-3 day old females per leaf disc collected 1,3, 7, 14 and 21 days after treatment	48-hour acute toxicity pesticide residue study: 3 apple trees sprayed at recommended application rate for Provado 2F 3 times in July or August. Insects evaluated 48-hours after exposure to leaf disk	Imidacloprid -treated leaves had no significant impact on mortality relative to controls at any of the sampling periods. Thus, imidacloprid residues are not harmful to *Colpoclypeus florus*	
Convergent lady beetle (*Hippondamia convergens*), 40 adults per concentration and acetone control	acute toxicity of technical grade imidacloprid (95% a.i.) At concentrations of 10, 50, 100, 200, 300 and 800 ppm in acetone applied topically to carbon dioxide-anesthetized beetles.	LD_{50} (95% confidence limits) in ug/g bw: 24-hour:1.8 (1.0 - 2.8) 48-hour:0.7(0.4 - 1.1) 72-hour: 0.4 (0.1 - 1.0)	Kaakeh et al. 1996

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Euonymus scale parasitoid hymenopteran (*Encarsia citrina*)	Effect of Marathon 60 WP on *Euonymus* scale and it's parasitoid *Encarsia citrina*. Soil drench at 0.33 g/500 ml water; foliar application at 0.15 g/500 ml of water	Both soil and Foliar application failed to control *Euonymus* scale with respect to untreated controls. Neither soil nor foliar application of Marathon 60 WP significantly impacted the number of parasitoids emerging from scale with respect to controls.	Rebek and Sadof 2003

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
parasitoid Hymenopteran (*Trichogramma nr. Brassicae*)	Confidor 350 SC (300 g/l a.i.). Applied at field application rate = 5.25 g a.i./100 L		Hewa-Kapuge et al. 2003
	single direct application to adults: 6-day assessment of mortality	100% mortality after 3 hours.	
20 -40 females per group of sprayed leaves.	residual exposure of adults: evaluation of mortality. potted tomato plants sprayed to runoff at label rate; wasps exposed to leaves 0, 1, 4 and 7 days after spraying	Significant increase in mortality (~60%) with respect to controls on day 0 only. Roughly 10-20% mortality on days 1, 4 and 7in comparison with a 0-5% control mortality on these days.	
15 females, tested in 3 groups of 5	residual exposure of adults: evaluation of ability to infect eggs for 24 hours	The number of eggs successfully parasitized did not differ significantly from untreated controls on days 0, 1, 3 and 7 following exposure	
5 replicates, 60 parasitized eggs each	exposure of life stages still inside host (egg or late pupal stages): parasitized Helicoverpa armigera eggs dipped in solutions for 1-2 seconds.	No difference between untreated controls and imidacloprid exposed host eggs for either egg or pupal life stages of wasp.	

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
parasitoid Ichneumonidae Hymenopteran (*Diadegma insulare*), 10 adults per treatment jar	LC_{50} test, leaf discs dipped in solutions of Provado 2F@ field application rate (0.22 mg ai/ml) and 0.01, 0.05, 0.1 and 0.5X. Leaves dipped in solution equal to a spray volume of 240 liter/ha, insects released to jars with treated leaf discs.	24-hour LC_{50}: 0.002 mg a.i./ml; 95% CI = 0.000 - 0.004 Given a spray volume of 240 l/ha, the LC_{50} of 0.002 mg ai/ml is = 0.00048 kg a.i./ha 2 mg ai/l x 240 L/ha = 480 mg ai/ha or 0.00048 kg a.i./ha	Hill and Fosler 2000
Hyaliodes vitripennis (predacious Mirid), 18 insects per concentration, 3 replicates, nymphs and adults tested seperately	24-hour acute toxicity test. Admire (240 g imidaclopid/L) sprayed on insect, apple leaf and sidewalls of plastic cage at concentrations in geometric progression of 1/256 X to X, where X = label application rate of 0.0312 g a.i./L	Nymph LC_{50}: 0.0023 g a.i./L, 95% CI: (0.0018 - 0.0029). Adult LC_{50} : 0.0011 g a.i./L, 95% CI: (0.0008 - 0.0017), The difference between adults and nymphs is not statistically significant. The LC_{50} values are lower than the label application rates, indicating use of the label application rate in the field would result in 100% mortality of both adult and nymph stages.	Bostanian et al. 2001

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Predatory bug *Orius laevigatus* (controls western flower thrips), 20 5th instar nymphs and 20 adults per concentration for each test	72-hour acute ingestion toxicity test with Confidor 200 SL (Imidacloprid a.i.), 8 concentrations 72-hour residual contact test with Confidor 200 SL (Imidacloprid a.i.), 5 concentrations	Nymph LC_{50}: 1.1 mg a.i./L (0.1 - 2.9) Adult LC_{50}: 2.1 mg a.i./L (1.0 -3.8) Nymph LC_{50}: 0.04 mg a.i./L (0.0002 - 1.2) Adult LC_{50}: 0.3 mg a.i./L (0.2 - 0.4) studies suggest that imidacloprid may be harmful to these predators.	Delbecke et al. 1997
Insidious flower bug *Orius insidiosus* (used on cotton), 8 days old, 6 males and 6 females per replicate, 6 replicates	72-hour acute toxicity. *Helicoverpa zea* eggs sprayed with Provado 1.6 flowable at 0.052 kg a.i. imidacloprid/ha compared with untreated controls	mortality, egg consumption and eggs laid (fecundity) were measured 72 hours after treatment. 47.8% and 62.7% mortality among males and females, respectively. Egg consumption and fecundity did not differ from control levels.	Elzen 2001
Trichogramma platneri (ectoparasitoid: attacks leafroller eggs) ,5 1-2 day old females	48-hour acute contact toxicity. Provado 2F sprayed on insects at label rate of "48 ppm or amount/100 gal."	100% mortality when applied at 100% label application rate for apple trees	Brunner et al 2001
Big-eyed bug *Geocoris punctipes,* (used on cotton) 8 days old, 6 males and 6 females per replicate, 6 replicates	72-hour acute toxicity. *Helicoverpa zea* eggs sprayed with Provado 1.6 flowable at 0.052 kg a.i. imidacloprid/ha compared with untreated controls	mortality and egg consumption were monitored 72 hours after treatment. 11.1% and 50.0% mortality among males and females, respectively. Egg consumption was significantly less than that of untreated controls.	Elzen 2001

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Predatory bugs (*Dicyphus tamaninii*) and (*Macrolophus caliginosus*), 3rd to 4th instar nypmhs, 10 nymphs per leaflet, 5 leaflets per treatment/control	Mortality 24h, 48h, and 7days after exosure to 1, 3, 8, 21 and 30-day residues of treated tomato leaflets. Also, evaluation of female reproductive capacity for 15 days . Imidacloprid, applied as Confidor 20LS (20% a.i.) applied at 0.5 ml/L (maximum recommended rate)	Both *D. tamaninii* and *M. caliginosus* nymphs died following exposure to imidacloprid-treated leaflets. *D. tamaninii* was more sensitive, with mortality ranging from 33.7% 24 hours after exposure to 1-day residues, to 91.9 % 7 days after exposure to 1-day residues. Percent mortality declined with increasing residue time, with 2 to 26.0% mortality at 24 hours and 7-days, respectively, after exposure to 30day residues.	Figuls et al. 1999

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Phytoseiid mite (*Amblyseius victoriensis*, used to control aphids in peach crops in Australia), 5-10 females per treated leaf disc, two leaf discs per treatment, test conducted 3 times	toxicity to adult females. Confidor 350 SC (5.25 g/100L or 0.0053% a.i.) sprayed on grape leaf discs at field rate to control aphids and 10X this rate	No mortality observed in controls or at field application rate. 34.4% mortality observed at 10X field rate	James 1997
50 females per grapefruit leaf platform, three platforms per treatment	12-day test for toxicity to eggs, Confidor 350 SC (0.0053% a.i.) Sprayed on leaves. Eggs recorded 12 days post-exposure.	Egg production in imidacloprid-exposed females (1.9 - 2.0eggs per female per day) was significantly increased with respect to untreated controls (1.3 - 1.6 eggs per female per day).	
Approximately 185 trees in imidacloprid-sprayed section of orchard; 185 trees in unsprayed section; 8 trees randomly selected from each section of analysis of leaves	Orchard study. Confidor 350 SC sprayed via label instructions at rate to control aphids (15 ml/100 L or 0.0053% a.i.)	Imidacloprid significantly reduced the population of *Amblyseius victoriensis* (beneficial phytoseiid mite) 4 weeks following application. However, the population recovered at 5-6 weeks following application, and was more than twice the size of the untreated control population (in another area of the orchard) by 9-12 weeks post-application	

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Vedalia beetle (*Rodolia cardinalis*), controls cottony cushy scale in citrus crops in California, 10-15 adults per replicate 3 replicates plus untreated controls;	72-hour contact-only- exposure to citrus (orange) leaves treated with imidacloprid (Provado 1.6 Flowable) either by soil drench (0.56 kg a.i./ha) or foliar spray application (0.14 kg a.i/ha). Leaves collected on 26, 35, 42, 51, 77 and 86 days post-treatment	48-hour post-treatment adult mortality and 7-day post-treatment assessment of emerged larvae and number of progeny per female beetle: foliar application significantly reduced adult survival and progeny per female 26 days after treatment. No significant impact when treatment was by soil drench.	Grafton-Cardwell and Gu 2003
10-15 second instar larvae per replicate, 3 replicates, cottony cushion scale larvae provided every 2-3 days	20-day contact only exposure to treated or untreated leaves, as above; larvae placed on scale-infested leaves 6 days after plants and scale were treated	larval mortality and stage of development evaluated every 2-3 days for 20 days exposure to treated or untreated leaves. No larvae survived in either treatment. All died within 2-3 days following exposure to leaves and insects treated by soil drench, and within 8 days following exposure to insects and leaves treated by foliar application	
15 adults per cottony cushion scale-infested, imidacloprid-treated or untreated branch, 2 branches per container, 3 containers per treatment. Same experiment	72-hour exposure to cottony cushion scale larvae raised on plants growing in imidacoprid-treated soil (0.15 ml, Admire 2F)	significantly reduced mean percentage of adult beetles and progeny with respect to controls on day 22 post-exposure, but not on days 43-155 post-exposure. Significantly reduced number of 2nd instar larvae surviving to adulthood (0 - 24.44% on days 8 - 29 after treatment; 51.11 - 66.67 on days 57 through	

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Earthworms and Nematodes			
Eisenia foetida, 6-10 worms per concentration	Acute toxicity of technical grade imidacloprid (>95% purity), with exposure via solution (distilled water control, 0.24, 0.48, 0.96, 2.00 mg/l), filter paper (acetone control, 0.004, 0.020, 0.100, 0.500 ug/cm^2) and artificial soil (control, 1,2,4,8, 16 mg/kg dry soil).	Solution: 24-hour LC_{50}: 1.23 mg/L 48-hour LC_{50}: 0.77 mg/L Filter Paper: 24-hour LC_{50}: 0.100 ug/cm^2 48-hour LC_{50}: 0.034 ug/cm^2 Artificial Soil: 7-day LC_{50}: 3.48 mg/kg dry soil 14-day LC_{50}: 2.30 mg/kg dry soil	Luo et al 1999; Zhang et al. 2000
Eisenia foetida, 3 worms per concentration	Comet assay for DNA damage. Exposure to technical grade imidacloprid (>95% purity) in 1% Tween-80 solution on ice at control, 5, 25, 50 and 100 mg/L for 2 hours	Extruded coelomocytes were examined for DNA damage to assess potential genotoxicity. DNA damage was significantly higher at all imidacloprid concentrations than in controls. The increase was dose-related	Zhang et al. 2000
Eisenia foetida, 6 worms per concentration	Test for sperm deformity with technical grade imidacloprid (> 95% purity) in artificial soil at 0, 0.1, 0.2, and 0.5 mg/kg dry soil for 10 days	Dose-related increase in sperm deformity. Statistically significant increase in percentage of deformed sperm with respect to controls at 0.2 and 0.5 mg/kg dry soil. NOAEC = 0.1 mg/kg dry soil	Luo et al 1999

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Entomopathogenic nematode *(Steinernema carpocapsae*), 2 ml of suspension (150 infective juveniles per ml) per concentration for mortality test, 0.05 ml suspension (180 - 200 infective juveniles per ml) for infectivity test	technical grade imidacloprid (90% purity). mortality test: 48-hour exposure to 0, 10 and 100 ug/ml in solution infectivity, development and reproduction test:100 ug/ml in solution for 24 hours	mortality test: no significant mortality in comparison with controls at any concentration tested. Infectivity, development, reproduction: nematodes treated with 100 ug/ml imidacloprid were no different than untreated controls in their ability to kill newly molted last instar cutworms (S. litura) in 3 trials conducted with 10 cutworms each treatment/control group.	Zhang et al. 1994
Entomopathogenic nematode (*Heterorhabditis bacteriophora*), 500 infective juveniles per ml	24-hour test of mortality and infectivity. Imidacloprid solutions at 0, 10, 40 or 160 mg a.i./l. 40 mg a.i. /l corresponds to the recommended field application rate of 400 g a.i/ha applied in 1 mm of water.	Imidacloprid did not affect nematode mortality with respect to controls. In addition, imidacloprid did not adversely impact the infective ability of nematodes (penetration of wax larvae of target moths) with respect to unexposed controls. In a separate greenhouse tests, imidacloprid was shown to act synergistically with the nematode in controlling white grubs in turfgrass.	Koppenhofer and Kaya 1998

Appendix 4: Toxicity of imidacloprid to terrestrial invertebrates

Species	Exposure	Effects [a]	Reference
Pheretima group earthworms (*Amynthas hawayanus, A. aeroginosus and A. diffringens*) Note: these are prevalent in South Africa, 10 worms per bucket, 5 buckets per concentration,	Commercially available imidacloprid formulation (350 g a.i./L) in artificial soil at 0, 3.5, 5.25, 7.0, 8.75, 10.50 mg a.i./kg soil (0, 10, 15, 20, 25, 30 ul 0.1 m^{-2})	all concentration in a.i.: 24-hour LC_{50}: 155 mg/kg soil (816 mg 0.1 m^{-2}); 48-hour LC_{50}: 5.0 mg/kg soil (26.3 mg 0.1 m^{-2}); 7-day LC_{50}: 3.0 mg/kg soil (15.8 mg 0.1 m^{-2}); These values are higher than the maximum application rate for the formulation: 1000 ml/ha (0.35 kg a.i./ha; 3.50 mg 0.1m^{-2})	Mostert et al. 2000

Species	Exposure	Effects [a]	Reference

Field Studies assessing multiple species

Kunkel et al. 1999. The effects of imidaclorpid and bendiocarb on beneficial invertebrates and predatory activity in turfgrass were evaluated.
Effects on earthworms and soil arthropods: Commercial formulations of Imidaclorpid (Merit 75 wettable powder and Merit 0.5% granular) were applied to plots of Kentucky bluegrass in Kentucky at the label-recommended rates for control of scarabaeid grubs. A randomized block design was used In a fall trial, with 5 replicate 2 x 2 m plots per formulation or untreated control. Merit 75 WP (applied via portable sprayer) and Merit 0.5G (applied via drop spreader) were tested at 0.34 kg a.i./ha. Merit 75 WP was also tested at 0.45 kg a.i./ha. Irrigation (1.5 cm) via lawn sprinkler was conducted after application. Impact on earthworms was determined 9 and 40 days post-application. Soil arthropods were sampled 15 days after application. Another identical test was conducted in the Spring, with the exception that earthworms were sampled on days 10 and 36 post-treatment. All imidacloprid treatments caused a temporary suppression in earthworm abundance in fall (40-50%), but only Merit 0.5G caused a reduction in abundance (39%) in spring. In both trials, earthworm abundance was no different than that of controls by the second sampling date (day 40 or 36for fall and spring, respectively). There was no effect of imidacloprid treatment on the abundance of soil micro-arthropods (Collembola, Mesostigmatid and Orbatid mites).

Effects on predatory arthropods and scarabaeid grubs: 2 successive trials (two different years) at two different golf courses in Kentucky. Merit 0.5G was applied in May, at a time when normally applied to control grubs, at a rate of 0.336 kg a.i./ha by drop spreader, followed by 1.5 cm irrigation. (2 sites, 10 x 10 m plots per treatment/control). Pre-treatment and pos-treatment arthropod samples were compared. There was no difference in pre-treatment counts for any group of predators in either year. Imidacloprid reduced the abundance of hister beetles and predatory larvae across all sample dates in 1996 but not in 1997. The abundance of beneficial predators (ants, carabids, spiders, and staphylinids) essentially was not impacted in either 1996 or 1997. Imidacloprid reduced scavenging rates on fresh-frozen black cutworms during the first week after treatment, but scavenging activity returned to normal with respect to controls 2-4 weeks post-treatment. There was no difference between controls and imidacloprid-treated plots with respect to scavenging of black cutworm eggs or Japanese beetle eggs. Ants were the primary predators.

Ants

Species	Exposure	Effects [a]	Reference

Zenger and Gibb 2001. Impact of imidacloprid on ant populations (predator) versus control of Japanese beetle eggs and grubs in Kentucky bluegrass was evaluated in Indiana. Imidacloprid was effective in controlling Japanese beetle eggs and white grubs, while not adversely affecting the ant population which preys on white grubs and eggs of Japanese beetles. In two separate trials, one in August and one in June, Merit granular applied at the maximum label application rate (0.34 kg a.i. imidacloprid/ha) to plots of turfgrass (6 replicate 10x10 plots), with irrigation, did not adversely affect the abundance of ants with respect to untreated control plots. Plots treated with imidacloprid had significantly fewer Japanese beetle eggs than control plots. Imidacloprid-treated plots had no grubs at all, in comparison with an average of 10.2 grubs per control plot.

Appendix 5: Toxicity of imidacloprid to fish and amphibians

Species	Exposure	Effects	Reference
Fresh Water Fish: Acute Toxicity			
Bluegill (*Lepomis machrochirus*), mean length 27mm, mean weight 0.46 g, 10 per concentration	Static 96-hour acute toxicity study with technical grade NTN 33893(97.4% a.i.). Control, solvent control (dimethylformamide), 16, 27, 45, 75 and 125 mg/L nominal concentrations equivalent to mean measured concentrations of control, solvent control, 14, 25, 42, 68 and 105 mg/L	96-hour LC_{50} > 105 mg/L (greater than the limit of solubility) 96-hour NOAEC = 25 mg/L 42 mg/L and higher: mortality, dark discoloration, fish on the bottom of test chamber, erratic swimming, surfacing, quiescence, rapid fin movement, labored respiration. A surface film and precipitate on the bottom were noted at these concentrations.	Bowman and Bucksath 1990a MRID 42055314
Rainbow Trout (*Ochorhynchus mykiss*), mean length 44 mm, mean weight 1.07 g, 10 per concentration	Static 96-hour acute toxicity study with technical grade NTN 33893(97.4% a.i.). Control, solvent control (dimethylformamide), 16, 27, 45, 75 and 125 mg/L nominal concentrations equivalent to mean measured concentrations of control, solvent control, 15, 27, 42, 64 and 83 mg/L	96-hour LC_{50} > 83 mg/L (greater than the limit of solubilty) 96-hour NOAEC = 42 mg/L 64 mg/L and higher: mortality, dark discoloration, fish on the bottom of test chamber, erratic swimming, quiescence. A surface film and precipitate on the bottom were noted at concentrations at and above 42 mg/L.	Bowman and Bucksath 1990b MRID 42055315

Appendix 5: Toxicity of imidacloprid to fish and amphibians

Species	Exposure	Effects	Reference
Rainbow Trout (*Salmo gairdneri*), mean length 5.3 cm, mean weight 1.3 g,, 10 per concentration.	Static 96-hour acute toxicity study with technical grade NTN 33893 (95.3% a.i.). Nominal concentrations of 0, 50, 89, 158, 281, 500 mg a.i./L, with measured greater than 80% of nominal values	96-hour LC_{50} = 211 mg a.i./L (158 - 281 mg a.i./L. 96-hour NOAEC = 50 mg a.i./L 89 mg/L and higher: apathy, irregular swimming behavior, lying on side/back, staggering 281 mg/L and higher: mortality	Grau 1988a MRID 42055316
Salt Fish: Acute Toxicity			
Sheepshead Minnow (*Cyprinodon variegatus*), young adult, mean length 29 mm, mean weight 0.77 g,, 10 per concentration	Static 96-hour acute toxicity test of technical grade NTN 33893(96.2% a.i.). Control, solvent control, 22.4, 35.2, 58.2, 105 and 195 mg/L mean measured concentrations	96-hour LC_{50} = 161 mg a.i./L, 95% CI = 105 - infinity, NOAEC = 58.2 mg a.i./L on the basis of mortality and signs (lethagy, dark coloration) at higher concentrations.	Ward 1990a MRID 42055318

Appendix 5: Toxicity of imidacloprid to fish and amphibians

Species	Exposure	Effects	Reference
Amphibians			
Rana linocharis tadpoles, 10 per concentration, 3 replicates per concentration	96-hour acute mortality study with >95% pure imidacloprid. 7 concentrations plus controls.	96-hour LC_{50} = 82 mg/L NOAEC = 16.7 mg/L LOAEC = 30 mg/L	Feng et al. 2004
Rana hallowell tadpoles, 10 per concentration, 3 replicates per concentration	96-hour acute toxicity study with >95% pure imidacloprid. 7 concentrations plus controls.	96-hour LC_{50} = 129 mg/L NOAEC = 67.5 mg/L LOAEC = 101.2 mg/L	
Supplemental information for Feng et al. 2004: *In vitro* micronucleus test conducted on tadpole erythrocytes (*Rana hallowell*), dose-related increase in chromosome damage, NOAEC = 2 mg/L, LOAEC = 8 mg/L. Comet Assay for DNA damage conducted on tadpole erythrocytes (*Rana hallowell*): significant difference from controls at all concentrations tested, LOAEC = 0.05 mg/L.			
Rana pipiens, Pseudacris triseriata, Ambystoma jeffersonianum, Bufo americanus, egg masses, approx. 70-100 eggs/mass, 3 replicates per concentration plus controls, except 2 replicates for *P. triseriata.*	study of hatching success and development. Egg masses from each species exposed to four imidacloprid concentrations based on previously reported LC_{50} values: control, 1.75-2.0 mg/l, 17.5 - 20 mg/L, and 88-110 mg/L	Previously reported LC50 values for ranids = approximately 176 - 220 mg/L No effects on hatching success. No significant differences between imidacloprid-exposed tadpoles and controls with regard to individual or total deformities. However, *P. triseriata* had a high and variable percentage of total deformities among controls (11.2%, 2.5 - 15%) which may have obscured a significant difference from high-dose tadpoles, which had a 24% mean rate (23-25%) of total deformity.	Julian and Howard 1999 MRID 44875001

Appendix 5: Toxicity of imidacloprid to fish and amphibians

Species	Exposure	Effects	Reference
Fish Chronic Toxicity			
Rainbow Trout (*Ochorhynchus mykiss*), newly fertilized eggs <4 hours old, 4 replicates of 35 eggs each per concentration, plus an additional 50 eggs per each of the 4 control replicates (egg viability determination)	98-Day flow-through early life stage test with technical grade NTN 33893 at nominal concentrations of 0, 1.3, 2.5, 5.0, 10 and 20 mg/L equivalent to mean measured concentrations of 0, 1.2, 2.3, 4.9, 9.8 and 19 mg/L	**original conclusions**: NOAEC = 9.8 mg/L LOAEC = 19 mg/L (statistically significant reduction in length at 36 and 60 days post-hatch, and body weight at 60 days post-hatch). No statistically significant biologically important effects on egg viability, hatch, survival or behavioral variables were observed. MATC (maximum acceptable toxicant concentration) = 14 mg/L (geometric mean of NOAEC and LOAEC) 1992 re-evaluation: Day 36 growth was most sensitive endpoint. Based on re-evaluation of this endpoint: NOAEC = 1.2 mg a.i./L LOAEC = 2.3 mg a.i./L MATC = 1.7 mg a.i./L	Cohle and Bucksath 1991 MRID 42055320 Gagliano 1992 MRID 42466501

Appendix 6: Toxicity of imidacloprid and imidacloprid metabolites to aquatic invertebrates

Species	Exposure	Effects	Reference
Fresh Water: Acute Toxicity			
Water Flea (*Daphnia magna*)., and Mosquito (*Aedes aegypti*) 3 trials, 4 replicates per concentration, 10 animals each species per replicate	Static 48-hour acute toxicity test. Technical grade imidacloprid (>95% purity)	Water Flea: 48-hour LC_{50} = 10.44 mg/L, 95% CI = 6.97 - 17.71 mg/L Mosquito: 48-hour LC_{50} = 0.044 mg/L, 95% CI = 0.041 - 0.047 mg/L	Song et al 1997; Song and Brown 1998
Water flea (*Daphnia magna*), 2 flasks per concentration with 10 each	Static 48-hour acute toxicity study with NTN 33893 (95.9% a.i.) at nominal concentrations up to 125 mg/L with actual mean concentrations of 0, 15, 25, 42, 71 and 113 mg/L	48-hour EC_{50} = 85 mg/L, 95% CI = 71 - 113 mg/L 48-hour NOAEC (immobility) = 42 mg/L Mobility was the endpoint of assessment	Young and Hicks 1990 MRID 42055317
Hyalella azteca (amphipod crustacean), 2-3 mm juveniles, 2 replicates per concentration, 10 per replicate	Static acute toxicity test with NTN 33893 at measured concentrations of control, 0.00035, 0.00097, 0.0035, 0.010, 0.034, 0.100, 0.340, 1.000 and 3.100 mg/L	96-hour LC_{50}: 0.526 mg/L, 95% CI = 0.194 - 1.263 mg/L 96-hour EC_{50} (immobilization): 0.055 mg/L, 95% CI = 0.034 - 0.093 mg/L 96-hour NOAEC (immobilization and abnormal effects, such as lethargy or surfacing) = 0.00035 mg/L	England and Bucksath 1991 MRID 42256303
Hyalella azteca (amphipod crustacean), 14 - 21 days old, two replicates per concentration, 10 organisms per replicate	96-hour static acute toxicity of NTN 33823 metabolite at mean measured concentrations of 0, 5.6, 11.0, 22.1, 43.8 and 86.8 mg/L	96-hour LC_{50}: 51.8 mg a.i/L, 95% CI = 44.0 - 60.9 mg a.i./L 96-hour EC_{50} (immobilization): 29.0 mg a.i./L, 95% CI = 24.7 - 34.0 mg a.i./L 96-hour NOAEC (mortality): 22.1 mg a.i./L	Rooney and Bowers 1996 MRID 43946601

Appendix 6: Toxicity of imidacloprid and imidacloprid metabolites to aquatic invertebrates

Species	Exposure	Effects	Reference
Hyalella azteca (amphipod crustacean), 7 - 21 days old, two replicates per concentration, 10 organisms per replicate	96-hour static acute toxicity of NTN 33519 **urea metabolite** at nominal (measured) concentrations of 0, 6.25 (5.81), 12.5 (11.80), 25 (23.46), 50 (46.80), and 100 (94.83) mg a.i./L	96-hour LC_{50}: > 94.83 mg a.i/L, 96-hour EC_{50} (immobilization): > 94.83 mg a.i/L, 96-hour NOAEC: 94.83 mg a.i./L	Dobbs and Frank 1996a MRID 43946603
Midge (*Chironomus tentans*), second instar, 2 replicates per concentration, 10chironomids per replicate	Static renewal 10-day toxicity test with technical grade NTN 33893 (95.0% a.i.) control, solvent control, measured concentrations of 0.00067, 0.00124, 0.00339, 0.0102, 0.0345, 0.100, and 0.329 mg a.i./L	96-hour LC50: 0.0105 mg/L, 95% CI = 0.0077 - 0.0144 mg/L 96-hour survival NOAEC: 0.00124 mg/L	Gagliano 1991 MRID 42256304
Midge (*Chironomus tentans*), 2 replicates per concentration, 10chironomids per replicate	96-hour static acute toxicity of **NTN 33823 metabolite** at mean nominal (measured) concentrations of 0, 0.1 (0.12), 1.0 (0.87), 10.0 (8.19) and 100 (82.8) mg a.i./L	96-hour LC_{50}: >82.8 mg a.i/L,96-hour EC_{50} (sub-lethal effects)): 17.0 mg a.i./L, 95% CI = 10.3 - 28.1mg a.i./L 96-hour NOAEC (mortality and sub-lethal effects): 8.19 mg a.i./L, sub-lethal effects included mottled coloration and erratic behavior.	Bowers 1996a MRID 43946602
Midge (*Chironomus tentans*), approximately 16 days old, 2 replicates per concentration, 10chironomids per replicate	96-hour static acute toxicity of **NTN 33519 urea metabolite** at nominal (measured) concentrations of 0, 0.1 (0.10), 1 (1.0), 10 (10.04) and 100 (99.80) mg a.i./L	96-hour LC_{50}: > 99.80 mg a.i/L, 96-hour EC_{50} (sub-lethal effects): >99.80 mg a.i/L, 96-hour NOAEC: 99.80 mg a.i./L	Dobbs and Frank 1996b MRID 43946604

Appendix 6: Toxicity of imidacloprid and imidacloprid metabolites to aquatic invertebrates

Species	Exposure	Effects	Reference
Midge (*Chironomus tentans*)	96-hour static acute toxicity of **6-chloronicotinic acid** (97% a.i.)	96-hour LC_{50}: > 1 mg a.i./L NOAEC = 1 mg a.i./L	Bowers and Lam 1988 MRID 44558901

Appendix 6: Toxicity of imidacloprid and imidacloprid metabolites to aquatic invertebrates

Species	Exposure	Effects	Reference
Fresh Water Invertebrates: Chronic Toxicity			
Water flea (*Daphnia magna*), 4 replicate jars per concentration, 6 first instar daphnids each jar	Chronic static renewal toxicity study of technical grade NTN 33893. Control, solvent control, 0.46, 0.86, 1.8, 3.6, and 7.3 mg/L	21-day EC_{50} (imobilization) >7.3 mg/L MATC = 2.5 mg/L (1.8 - 3.6 mg/L) NOAEC = 1.8 mg/L LOAEC = 3.6 mg/L 3.6 and 7.3 mg/L: Significantly reduced adult daphnid length in comparison with pooled controls 7.3 mg/L: Significantly reduced survival; significantly reduced mean young/adult reproduction days in comparison with pooled controls. No effects on time to first brood at any concentration	Young and Blake 1990 MRID 42055321
Freshwater: Mesocosm			
Multiple-species: phytoplankton, zooplankton, macroinvertebrates, including *Hyalella azteca*; 3 tanks each for control and 5 concentration levels	19-Week microcosm study with technical grade NTN 33893 (95.8% a.i.): Four surface applications at 2- week intervals at nominal concentrations of 0, 0.002, 0.006, 0.020, 0.060 and 0.180 mg a.i./L, with average measured concentrations of 0, 0.0015, 0.0047, 0.019, 0.058 and 0.180 mg a.i./L	Half-life of NTN 33893 in water: 1.4 days Minimal partitioning to sediment: rapid degradation of residues which partition to sediment, with residues non-detectable 2 weeks after last application. No effects of NTN 33893 on temperature stratification, dissolved oxygen, pH. ***Continued below:***	Moring et al. 1992 MRID 42256306

Species	Exposure	Effects	Reference

Moring et al. 1992 (continued) – Amphipods were the most sensitive species, with statistically significant impacts at the lowest concentration tested. Impacts (statistically significant decrease in population) on cyanophytes (blue-green algae) and copepods at the 3 highest doses. Statistically significant decrease in populations of total macroinvertebrates as well as individual macroinvertebrate taxa (Mayfly, Midge, Caddisfly, Beetle and Amphipod populations were most affected) at the three highest doses. Study authors recommend 0.006 mg/L as NOEC for regulatory action. However, on basis of total macroinvertebrates and macroinvertebrate taxonomic richness, the overall NOAEC is 0.002 mg/L. On the basis of amphipod sensitivity, the LOAEC is 0.002 mg/L.

Appendix 6: Toxicity of imidacloprid and imidacloprid metabolites to aquatic invertebrates

Species	Exposure	Effects	Reference
Salt Water: Acute Toxicity			
Artemia sp., and Mosquito (*Aedes taeniorhynchus*) 3 trials, 4 replicates per concentration, 10 animals each species per replicate	Static 48-hour acute toxicity test. Technical grade imidacloprid (>95% purity)	Artemia: 48-hour LC_{50} = 361.23 mg/L, 95% CI = 307.83 - 498.09 mg/L Mosquito: 48-hour LC_{50} = 0.13 mg/L, 95% CI = 0.010 - 0.016 mg/L Note: increasing salinity increased sensitivity to imidacloprid	Song et al 1997; Song and Brown 1998
Mysid (*Mysidopsis bahia*), < 24 hours old, 10 per concentration.	96-hour flow-through acute toxicity tests of technical grade NTN 33893 (96.2% a.i.). Mean measured concentrations: First test: control, solvent control, 0.032, 0.0584, 0.0937, 0.146 and 0.249 mg a.i./L Second test: control, solvent control, 0.00842, 0.0133, 0.0229, 0.0372 and 0.0634 mg a.i./L	First test: 96-hour LC_{50} = 0.0377 mg a.i./L, 95% CI = 0.0267 - 0.0464 mg a.i./L, NOAEC not determined. Second test: 96-hour LC_{50} = 0.0341 mg a.i./L, 95% CI = 0.0229 - 0.0372 mg a.i./L, NOAEC = 0.0133 mg a.i./L on the basis of mortality and loss of equilibrium at higher doses.	Ward 1990b MRID 42055319
Mysid (*Mysidopsis bahia*), < 24 hours old, 2 replicates per concentration, 10 per replicate	96-Hour flow-through acute toxicity test, NTN 33893 240 FS Formulation, control, solvent control, 18 (21), 29 (31), 49 (56), 82 (78), 136 (125) and 227 (219) ug a.i./L nominal (measured) concentrations	96-hour LC_{50} = 0.036 mg a.i./L, 95% CI = 0.031 - 0.042 mg a.i./L NOAEC (mortality) = 0.021 mg a.i./L	Lintott 1992 MRID 42528301

Appendix 6: Toxicity of imidacloprid and imidacloprid metabolites to aquatic invertebrates

Species	Exposure	Effects	Reference
Eastern Oyster (Crassostrea virginica), 20 per concentration	96-hour flow-through test of effect on shell growth. Technical grade NTN 33893 (95.8% and 96.2% a.i. for second and first tests, respectively) First test: control, solvent control, 2.93, 5.14, 8.19, 14.2, and 23.3 mg a.i./L, measured Second test: control, 145.0 mg a.i./L, measured	First test: 100% survival; No effects on new shell growth Second test: 100% survival; new shell growth of exposed was 22% less than controls. This was statistically significant. 96-hour NOAEC: 145 mg/L	Wheat and Ward 1991 MRID 42256305
Saltwater: Chronic Toxicity			
Midge (*Chironomus tentans*), second instar, 2 replicates per concentration, 10chironomids per replicate	Static renewal 96-hour toxicity test with technical grade NTN 33893 (95.0 % a.i.) control, solvent control, measured concentrations of 0.00067, 0.00124, 0.00339, 0.0102, 0.0345, 0.100, and 0.329 mg a.i./L	10-day LC_{50}: 0.00317 mg/L, 95% CI = 0.00124 - 0.0102 mg/L 10-day survival NOAEC: 0.00124 mg/L 10-day growth NOAEC: 0.00067 mg/L (basis = dry weight of survivors)	Gagliano 1991 MRID 42256304

Appendix 6: Toxicity of imidacloprid and imidacloprid metabolites to aquatic invertebrates

Species	Exposure	Effects	Reference
Mysid (Mysidopsis bahia), <24-hours old, 4 replicates per concentration, 15 mysids per replicate cup	Flow-through chronic toxicity tests with technical grade NTN 33893 (96.2% a.i.) First test: control, solvent control, 560, 1290, 2850, 5080 and 10100 ng a.i./L mean measured Second test: control, solvent control, 36.8, 78.4, 163, 326 and 643 ng a.i./L nominal	**First Test**: 1290 ng/L and higher: Significantly reduced number of offspring per female reproductive day 5080 ng/L and higher: significantly reduced growth of first generation mysids as total length and as dry weight 10,100 ng/L: Statistically increased mortality in comparison with pooled controls for first generation. No effects on mortality in second generation MATC (reproductive success): 849 ng/L (560 - 1290 ng/L) MATC (growth): 3806 ng/L (2850 - 5080 ng/L) **Second Test**: No effects on number of offspring per female reproductive day. 326 and 643 ng/L: Significantly reduced growth of first generation as total length and as dry weight in comparison with pooled controls 643 ng/L: Statistically increased mortality in comparison with pooled controls for first generation. No effects on mortality in second generation. MATC (reproductive success): > 643 ng/L MATC (growth): 230 ng/L (163 - 3260 ng/L) No real explanation for discrepancy between first and second tests with regard to growth.	Ward, 1991 MRID 42055322

Appendix 7: Toxicity of imidacloprid to aquatic plants

Species	Exposure	Effects	Reference
Blue-Green Algae (*Anabaena flos-aquae*)	NTN 33893 2F (21.6% a.i.) at mean measured concentrations of 0, 24.9, 40.5, 68.2, 121.3, and 193.3 mg a.i./L.	4-Day EC_{25} = 26.7(18.9-29.2) mg a.i./L 4-Day EC_{50} = 32.8(30.4-34.6) mg a.i./L 4-Day NOEC = 24.9 mg a.i./L	Bowers 1996b MRID 44187101
Diatom (Navicula pelliculosa)	acute toxicity of NTN 33893 2F (21.6% a.i.), mean measured concentrations: control, 0.16, 0.42, 1.05, 2.64, 6.69, and 17.0 mg a.i./L	4-day NOAEC: 6.69 mg a.i./L 4-day LOAEC: 9.88 mg a.i./L	Hall 1996 MRID 44187102
Green algae (*Scenedesmus subspicatus*)	acute toxicity, technical grade NTN 33893 (92.8% a.i.) at nominal concentrations of 0, 0.1, 1, and 10 mg a.i./L	72- and 96-hour EC_{50} (biomass and growth): > 10 mg a.i./L 72- and 96-hour NOAEC biomass and growth: 10 mg a.i./L	Heimbach 1989 MRID 42256374
Green algae (*Selanastrum capricornutum*)	acute toxicity, technical grade NTN 33893 (95% a.i.) at nominal (measured) concentrations of 0, 15.6(14.1), 25.9 (24.1), 43.2 (41.1), 72v(69.5), and 120 (119) mg a.i./L	5-day EC_{50} (biomass/growth): >119 mg/L 5-day NOAEC: Test limits: > 119 mg/L	Gagliano and Bowers 1991 MRID 42256374

Data Summary	Reference
Aquatic Sediment Halftimes	
Anaerobic halftime of 27 days	Fritz and Hellpointner 1991, MRID 42256378
Hydrolysis	
As Confidor formulation: 33.82 to 41.2 days at pH 7 As Gaucho formulation: 37.6 days to 44.26 days Note: The reported halftimes are possibly a combination of hydrolysis and photolysis. Cannot determine lighting (if any) from methods.	Sakar et al. 1999
stable (pH 5) stable (pH 7) 355 days (pH 9)	Yoshida 1989, MRID 42055337
Only 1.5 % loss in three months at pH 7. 20 days (pH 10.8) 2.85 days (pH 11.8)	Zheng and Liu 1999
Photolysis, Aqueous	
Environmental halftime of 4.2 hours at pH 7 based on experimental halftime of 57 minutes.	Anderson 1991, MRID 42256376
Experimental halftime of 1.2 hours at 290 nm for 4 hours.	Moza et al. 1998
Imidacloprid as a.i. in HPLC water: 43 minutes. Confidor formulation in tap water: 126 minutes.	Wamhoff and Schneider 1999
Photolysis, Soil	
38.9 days	Yoshida 1990, MRID 42256377
460 hours (19 days) in moist soil 830 hours (34.6 days) in dry soil [bi-phasic pattern]	Graebing and Chib 2004
Soil Degradation/Dissipation	
Halftime of > 1 year in anaerobic soil with no light.	Anderson et al. 1991 MRID 42073501
After application as Conifer formulation: 39 days with range of 27.8 to 44.9 days. After application as Gaucho formulation: 40.7 days with range of 35.8 to 46.3 days.	Sarkar et al. 2001

Data Summary	Reference
Soil Binding (Kd, Ko/c)	
Greater binding at lower concentrations: Koc of 77 at half of water solubility and 411 at field application rate.	Cox et al. 1997
Fine Sand (0.29% OC): Kd 0.52 (Ko/c 179) Silty clay loam (3.95% OC): Kd 11.4 (Ko/c 288) Fine sandy loam (0.41% OC): Kd 0.40 (Ko/c 98) Sandy loam (0.7% OC): Kd 3.40 (Ko/c 487) Silty clay (1.34% OC): Kd 3.10 (Ko/c 228) Silt loam (2..5% OC): Kd 5.7 (Ko/c 228) Silty clay loam (1.05% OC): Kd 4.8 (Ko/c 454) Above are from Table 1 (p. 125) and Table 2 (p. 128) in publication. Soil sorption is concentration dependent (greater at lower concentrations) and OC is major factor in sorption. Very low leaching potential.	Cox et al. 1998a,b
Kd values in salt water sediment of 0.28 to 0.62.	Felsot and Ruppert 2002
Kd 3.59 in low humus sandy soil Kd 2.39 in silt soil Kd 1.36 in silty clay soil	Fritz 1988, MRID 42055338
Calcium Montmorillonite Kd 6.86 Humic acid Kd 247 at 1:200 Humic acid Kd 326 at 1:100 Binding to clay inhibited by humic acid (competitive)	Liu et al. 2002
Kd 1.43, Ko/c 209.6 in clay alluviation (0.68 % OC)	Nemeth-Konda et al. 2002
4.82 on Day 0 and 15.6 on Day 100 in sandy loam (1.8%OC) 2.24 on Day 0 and 8.6 on Day 100 in silt loam (0.9% OC) Greater binding (decreased leaching) over time. *"It is concluded that increasing Koc values are mainly due to change in the sorption process leading to stronger sorption to soil, thereby persistence in soil. These results are further information to explain the gap between the estimated leaching potential of imidacloprid from conventional laboratory experiments and field data. These factors should be taken into account when the potential mobility of imidacloprid in soil is evaluated."* (p. 331, last paragraph).	Oi 1999

Appendix 8: Laboratory studies on the environmental fate of imidacloprid

Data Summary	Reference
Kd 11.3, Ko/c 779 in clay (1.45% OC) Kd 0.55, Ko/c 158 in loamy sand (0.35% OC) Kd 5.18, Ko/c 186 in clay (2.78% OC) Kd 1.18, Ko/c 203 in sand (0.58% OC) Kd 16.9, Ko/c 227 in sandy loam (7.45% OC) Kd 10.8, Ko/c 620 in sand clay loam (1.74% OC) Higher sorption with decreasing concentration in parent and metabolites indicating low soil mobility.	Oliveira et al. 2000
Kd 0.956, Ko/c 411 in sand (0.233 % OC) Kd 1.02, Ko/c 292 in loamy sand (0.349 % OC) Kd 4.18, Ko/c 277 in silt loam (1.51 % OC) Kd 3.45, Ko/c 296 in loam (1.16 % OC)	Williams et al. 1992a, MRID 42520801 Williams et al. 1992b, MRID 42520802 *These appear to be duplicate submissions but they have different report numbers.*

Appendix 9: Summary of field or field simulation studies on the environmental fate of imidacloprid.

Application	Observations	Reference
Turf plots with 5% slope. Granule and liquid formulations. 5 cm (2 inches) of simulated rainfall 24 hours after application.	0.95% runoff after rainfall simulation for WP formulation. 1.47 % runoff after rainfall simulation for granular formulation.	Armbrust and Peeler 2002
Field applications to bare soil (loam to sandy loam with OM of 1.36 to 3.82%) at 0.17 kg/ha (0.15 lb/acre). Irrigated at 300 L/ha.	Soil dissipation halftimes of 79 to 196 days. No mobility below 0 to 10 cm (3.9 inches).	Bachlechner 1992, MRID 42734101
Applied to fine sandy loam (3.2% clay and 1.03 OM). Drip chemigation at a depth of 38 to 45 cm. Application rate not clear.	Significant leaching because of lack of coordination of irrigation timing with soil moisture, creating near saturated conditions. Imidacloprid penetrated down to 100 cm (about 39 inches).	Felsot et al. 1984
Leaching studies with imidacloprid and a lignin granular formulation. (Not clear if this formulation is used commercially). Actual concentrations or application rates not clear.	Substantially less leaching potential for lignin granular formulation.	Fernandex-Perex et al. 1998
Laboratory leaching studies	Dissolve organic carbon in soil (e.g., from augmentation of low OC soils) may competitively reduce the binding of imidacloprid to soil and enhance the potential for leaching.	Flores-Cespedes et al. 2002
Standard leaching study using ^{14}C-labeled imidacloprid in sandy loam soil. Incorporated into soil at maximum commercial use rate, 0.38 ppm.	Relatively immobile **after aging for 30 days**. After irrigation of soil column with the equivalent of 20 inches of rain, 48.5% remained in top layer. Only 0.1% leached to the 25-30 cm layer. Note: This or a very similar study is summarized and discussed in Krohn and Hellpointner (2002) but not specifically referenced.	Fritz and Brauner 1988, MRID 42055339

Appendix 9: Summary of field or field simulation studies on the environmental fate of imidacloprid.

Application	Observations	Reference
Soil column leaching equivalent to rainfall of 65 cm (25.6 inches). Sandy loam (0.864% OM), saturate flow with 2 cm water head. Used t.g.a.i., Gaucho 70 WS, Confidor 200 SL, and Admire 250 SC formulations. Initial concentration in soil of 1 mg/10 g [0.01 mg/kg] or 0.01 ppm.	Detectable residues at depth of up to 25 cm (total depth of column). Greater leaching with formulations compared to t.g.a.i. Greatest concentration of imidacloprid in 20-25 cm layer. Approximately 26% to 29% of the imidacloprid leached through the soil column. Greater leaching of formulations (32% to 44.5%) attributed to adjuvants (speculative). *Note: Soil concentrations in various fractions are reported in the range of 0.2 to 0.8 ppm. This is not consistent with methods – i.e., 10 g of soil with a concentration of 0.01 ppm.*	Gupta et al. 2002
Lysimeter study using ^{14}C-labeled imidacloprid in sandy loam soil. Application rate equivalent to 0.52 kg/ha (0.46 lb/acre).	No leaching over a 2 year observation period. About 40% lost over study period, presumably due to mineralization. About 55% of the applied radioactivity was recovered from the soil at the end of 2 years.	Hellpointner 1994a MRID 43142501 Hellpointner 1994b MRID 43315201
Applied to turf at a rate of 0.5 lb/acre.	Initial residues of 40 to 45 ppm (consistent with Fletcher et al. 1994 default of 85 ppm at 1 lb/acre for short grass). **Foliar dissipation halftime of 9.8 days.**	Lin 1992a, MRID 42256307 Lin 1992c, MRID 42488101
Applied to potato foliage at a rate of 0.5 lb/acre.	Initial residues of 2 to 4 ppm (consistent with Fletcher et al. 1994 default of 7 ppm at 1 lb/acre for fruits). **Foliar dissipation halftime of 1.17 days.**	Lin 1992d, MRID 42556101
Applied to turf (silty clay loam soil) at a rate of 0.5 lb/acre and then irrigated with 2 and 3.5 inches of water.	56% to 71% loss in runoff from turf.	Lin 1992b, MRID 42256309

Appendix 9: Summary of field or field simulation studies on the environmental fate of imidacloprid.

Application	Observations	Reference
Residues on crops after soil applications at rates of 0.29 to 0.32 lb/acre.	Residues of 0.12 ppm in wheat, 0.58 ppm in turnip tops, and 0.32 ppm in leafy crops. These correspond to residue rates of about 0.4 ppm, 1.9 ppm, and 1 ppm per lb/acre. These rates are much less than rates after foliar application.	Minor 1994, MRID 43245901
Field dissipation study on bare sandy loam soil applied at a rate of 0.5 lb/acre	Dissipation halftime of 12 days with a total rainfall of 78.5 inches and irrigation of 15.83 inches. No residues below 6 inches in soil column. Very low potential for leaching.	Rice et al. 1991a, MRID 42256379
Applied to field corn (sandy loam soil) at a rate of 0.5 lb/acre.	Field halftime of 7 days. No residues below 6 inches. Total rainfall of 57.17 inches and irrigation of 4.18 inches.	Rice et al. 1991b, MRID 42256380
Applied to tomatoes (sandy loam soil) at a rate of 0.5 lb/acre.	Field halftime of 53 days. No residues below 6 inches. Total rainfall of 9.25 inches and irrigation of 51.43 inches.	Rice et al. 1991c, MRID 42256381
Applied to turf (loamy sand soil) at a rate of 0.5 lb/acre.	Field halftime of 107 days. No residues below 3-6 inches. Total rainfall of 8.30 inches and irrigation of 7.78 inches.	Rice et al. 1992a, MRID 42256382
Applied to turf (loam soil) at a rate of 0.5 lb/acre.	Field halftime of 61 days. No residues below 3-6 inches. Total rainfall of 22.13 inches. No irrigation.	Rice et al. 1992b, MRID 42256383
Field trials on various crops with and without fertilizer.	Fertilizer applied with pesticide increased persistence in soil due to slow release from the added fertilized (OC adsorption).	Rouchaud et al. 1996
Application of 0.4 lbs/acre to turf	Residues on turf of 42 ppm, very similar to Lin 1992a. **Turf halftime of 4.5 days**, also similar to Lin 1992a. Residues on terrestrial invertebrates estimated at 6.38 ppm or about 16 ppm per lb/acre. This is in the range of estimates from Fletcher et al. (1994) with default values of 7 ppm to 15 ppm at 1 lb/acre for large insects.	Toll 1994, MRID 43472301

Appendix 9: Summary of field or field simulation studies on the environmental fate of imidacloprid.

Application	Observations	Reference

Appendix 10: Summary of GLEAMS Modeling of Broadcast Application of a Liquid Formulation of Imidacloprid to a Two Acre Plot

Table 1: Summary of modeled concentrations in streams (all units are ug/L or ppb per lb/acre applied) [**Strm01**]

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0	0	0	0	0	0
10	0	0	0	0	0	0
15	0.0094	0.437	0	0	0	0
20	0.0214	1.06	0	0	0	0
25	0.0355	1.87	0	0	7.85E-09	6.68E-07
50	0.105	7.27	0.000118	0.0169	0.00631	0.0901
100	0.183	19.9	0.00855	1.76	0.0446	0.928
150	0.208	31.7	0.0157	3.79	0.0803	2.01
200	0.212	42.2	0.0203	5.41	0.102	3.08
250	0.207	51.4	0.0234	6.64	0.113	3.93

Table 2: Summary of modeled concentrations in ponds (all units are ug/L or ppb per lb/acre applied) **[Pond01]**

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0	0	0	0	0	0
10	0	0	0	0	0	0
15	0.183	0.624	0	0	0	0
20	0.287	1.05	0	0	0	0
25	0.387	1.82	0	0	6.67E-08	3.24E-07
50	0.72	6.39	0.000888	0.0122	0.0328	0.0797
100	0.952	15.3	0.049	1.14	0.163	0.871
150	0.994	23.1	0.0818	2.59	0.253	1.74
200	0.985	28.8	0.102	3.95	0.304	2.68
250	0.961	37.2	0.115	5.13	0.329	3.48

Table 3: Summary of modeled concentrations in the entire 60 inch soil column (all units are mg/kg soil or ppm per lb/acre applied)**[Soil]**

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0.00851	0.0375	0.0071	0.0343	0.00689	0.0343
10	0.00926	0.0376	0.00807	0.0345	0.00724	0.0343
15	0.00903	0.0375	0.00785	0.0344	0.00733	0.0344
20	0.00874	0.0372	0.00778	0.0344	0.00738	0.0344
25	0.00841	0.037	0.00769	0.0344	0.00751	0.0346
50	0.00669	0.0352	0.00756	0.0344	0.00841	0.035
100	0.00419	0.0305	0.0075	0.034	0.0086	0.0344
150	0.00276	0.0263	0.00745	0.0334	0.00784	0.0342
200	0.00189	0.0262	0.0074	0.0327	0.00698	0.0342
250	0.00131	0.0262	0.00733	0.032	0.00622	0.0342

Table 4: Summary of modeled concentrations in the top 12 inches of the soil column (all units are mg/kg soil or ppm per lb/acre applied)**[Soil12]**

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0.0425	0.187	0.0355	0.172	0.0344	0.172
10	0.0463	0.188	0.0404	0.172	0.0362	0.172
15	0.0451	0.187	0.0393	0.172	0.0362	0.172
20	0.0437	0.186	0.0389	0.172	0.0348	0.171
25	0.042	0.185	0.0385	0.172	0.0332	0.171
50	0.0335	0.176	0.0371	0.172	0.0257	0.169
100	0.0209	0.153	0.0342	0.169	0.0168	0.16
150	0.0138	0.131	0.0318	0.164	0.0125	0.149
200	0.00944	0.131	0.0299	0.16	0.00994	0.138
250	0.00655	0.131	0.0284	0.156	0.00831	0.129

Table 5: Summary of modeled maximum depth of chemical in the soil column and days to maximum ()**[SoilMaxDepth]**

Annual Rainfall (inches)	Clay		Loam		Sand	
	Depth	Julian Day	Depth	Julian Day	Depth	Julian Day
5	6.5	1991181	6.5	1991181	6.5	1991181
10	6.5	1991181	6.5	1991181	6.5	1991181
15	12	1991271	18	1992001	30	1991311
20	12	1991211	18	1991191	36	1991251
25	12	1991191	18	1991181	48	1992001
50	12	1991181	30	1991251	60	1991241
100	12	1991181	42	1992001	60	1991181
150	12	1991181	48	1992011	60	1991181
200	12	1991181	54	1992021	60	1991181
250	12	1991181	60	1992071	60	1991181

Table 6: Summary of the cumulative loss from soil runoff and sediment as a proportion of the application rate **[PropRunoSed]**

Annual Rainfall (inches)	Clay	Loam	Sand
5	0	0	0
10	0	0	0
15	0.014	0	0
20	0.0317	0	0
25	0.052	0	0
50	0.148	0.000214	0
100	0.265	0.0192	0
150	0.318	0.0396	0
200	0.344	0.0564	0
250	0.357	0.0704	5.02E-08

Appendix 11: Summary of GLEAMS Modeling of Broadcast Application of a Granular Formulation of Imidacloprid to a Two Acre Plot

Table 1: Summary of modeled concentrations in streams (all units are ug/L or ppb per lb/acre applied) [**Strm01**]

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0	0	0	0	0	0
10	0	0	0	0	0	0
15	0.013	0.604	0	0	0	0
20	0.0295	1.46	0	0	0	0
25	0.0491	2.59	0	0	1.61E-08	8.34E-07
50	0.145	10.1	0.000156	0.0223	0.00844	0.12
100	0.253	27.5	0.0113	2.32	0.0594	1.23
150	0.288	43.8	0.0207	5	0.107	2.67
200	0.293	58.3	0.0269	7.15	0.136	4.09
250	0.286	71.1	0.031	8.77	0.15	5.24

Table 2: Summary of modeled concentrations in ponds (all units are ug/L or ppb per lb/acre applied) **[Pond01]**

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0	0	0	0	0	0
10	0	0	0	0	0	0
15	0.253	0.862	0	0	0	0
20	0.397	1.46	0	0	0	0
25	0.535	2.52	0	0	1.67E-07	4.54E-07
50	0.996	8.83	0.00117	0.0161	0.0441	0.107
100	1.32	21.2	0.0648	1.5	0.218	1.16
150	1.37	31.9	0.108	3.42	0.337	2.31
200	1.36	39.8	0.135	5.22	0.405	3.56
250	1.33	51.5	0.153	6.78	0.438	4.63

Table 3: Summary of modeled concentrations in the entire 60 inch soil column (all units are mg/kg soil or ppm per lb/acre applied)**[Soil]**

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0.0118	0.0528	0.00949	0.0464	0.0092	0.0464
10	0.0129	0.053	0.0108	0.0467	0.00967	0.0464
15	0.0126	0.0529	0.0105	0.0466	0.0098	0.0465
20	0.0122	0.0528	0.0104	0.0465	0.0099	0.0466
25	0.0117	0.0528	0.0103	0.0465	0.0101	0.0468
50	0.00932	0.0526	0.0101	0.0466	0.0112	0.0474
100	0.00586	0.0525	0.01	0.0468	0.0115	0.0465
150	0.00389	0.0525	0.00997	0.0469	0.0105	0.0463
200	0.00268	0.0524	0.00992	0.047	0.00934	0.0463
250	0.00188	0.0524	0.00988	0.0471	0.00833	0.0463

Table 4: Summary of modeled concentrations in the top 12 inches of the soil column (all units are mg/kg soil or ppm per lb/acre applied)**[Soil12]**

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0.0591	0.264	0.0475	0.232	0.046	0.232
10	0.0643	0.265	0.0539	0.233	0.0484	0.232
15	0.0628	0.264	0.0526	0.233	0.0483	0.232
20	0.0608	0.264	0.052	0.233	0.0465	0.232
25	0.0585	0.264	0.0514	0.233	0.0444	0.232
50	0.0466	0.263	0.0496	0.232	0.0344	0.231
100	0.0293	0.263	0.0457	0.232	0.0227	0.231
150	0.0194	0.262	0.0425	0.232	0.0169	0.231
200	0.0134	0.262	0.0401	0.232	0.0135	0.231
250	0.0094	0.262	0.0381	0.231	0.0113	0.231

Table 5: Summary of modeled maximum depth of chemical in the soil column and days to maximum ()**[SoilMaxDepth]**

Annual Rainfall (inches)	Clay		Loam		Sand	
	Depth	Julian Day	Depth	Julian Day	Depth	Julian Day
5	6.5	1991181	6.5	1991181	6.5	1991181
10	6.5	1991181	6.5	1991181	6.5	1991181
15	12	1991231	18	1992001	30	1991261
20	12	1991201	18	1991191	42	1992051
25	12	1991191	24	1992001	48	1992001
50	12	1991181	30	1991231	60	1991231
100	12	1991181	42	1991311	60	1991181
150	12	1991181	48	1991331	60	1991181
200	12	1991181	60	1993081	60	1991181
250	12	1991181	60	1992021	60	1991181

Table 6: Summary of the cumulative loss from soil runoff and sediment as a proportion of the application rate **[PropRunoSed]**

Annual Rainfall (inches)	Clay	Loam	Sand
5	0	0	0
10	0	0	0
15	0.0194	0	0
20	0.0438	0	0
25	0.0719	0	0
50	0.205	0.000283	0
100	0.366	0.0254	0
150	0.439	0.0523	0
200	0.475	0.0745	0
250	0.493	0.0931	6.58E-08

Appendix 12: Summary of GLEAMS Modeling For Soil Injection of Imidacloprid Adjacent to a 1 Acre Plot Along a Body of Surface Water (Stream or Pond)

Table 1: Summary of modeled concentrations in streams (all units are ug/L or ppb per lb/acre applied) [**Strm01**]

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0	0	0	0	0	0
10	0	0	0	0	0	0
15	0	0	0	0	0	0
20	0	0	0	0	0	0
25	0	0	0	0	1.09E-08	5.91E-07
50	0	0	6.63E-08	1.91E-06	0.00467	0.0661
100	0	0	2.10E-06	9.46E-05	0.0322	0.666
150	0	0	3.97E-07	8.22E-05	0.0583	1.46
200	0	0	3.47E-06	6.08E-05	0.0746	2.25
250	0	0	3.07E-05	0.000829	0.0829	2.91

Table 2: Summary of modeled concentrations in ponds (all units are ug/L or ppb per lb/acre applied) **[Pond01]**

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0	0	0	0	0	0
10	0	0	0	0	0	0
15	0	0	0	0	0	0
20	0	0	0	0	0	0
25	0	0	0	0	1.49E-07	4.00E-07
50	0	0	5.52E-07	2.81E-06	0.0333	0.0747
100	0	0	1.24E-05	0.000121	0.156	0.783
150	0	0	1.67E-06	4.33E-05	0.24	1.55
200	0	0	6.45E-06	1.42E-05	0.285	2.38
250	0	0	4.34E-05	0.000118	0.308	3.09

Table 3: Summary of modeled concentrations in the entire 60 inch soil column (all units are mg/kg soil or ppm per lb/acre applied)**[Soil]**

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0.00976	0.0525	0.00846	0.0464	0.00873	0.0464
10	0.0116	0.0528	0.0108	0.0468	0.00868	0.0464
15	0.011	0.0527	0.00951	0.0465	0.00943	0.0465
20	0.0113	0.0527	0.00954	0.0465	0.00972	0.0467
25	0.0115	0.0527	0.00961	0.0465	0.00999	0.0468
50	0.0122	0.0529	0.00986	0.0466	0.0113	0.0475
100	0.013	0.053	0.0103	0.0468	0.0116	0.0465
150	0.0134	0.0531	0.0106	0.0471	0.0105	0.0463
200	0.0136	0.0532	0.0109	0.0472	0.00934	0.0463
250	0.0137	0.0533	0.0111	0.0474	0.00832	0.0463

Table 4: Summary of modeled concentrations in the top 12 inches of the soil column (all units are mg/kg soil or ppm per lb/acre applied)**[Soil12]**

Annual Rainfall (inches)	Clay		Loam		Sand	
	Average	Maximum	Average	Maximum	Average	Maximum
5	0.0488	0.263	0.0423	0.232	0.0437	0.232
10	0.0578	0.264	0.054	0.234	0.0434	0.232
15	0.0552	0.263	0.0476	0.232	0.0462	0.232
20	0.0563	0.263	0.0477	0.232	0.0447	0.232
25	0.0574	0.264	0.0477	0.233	0.0427	0.232
50	0.0612	0.264	0.0472	0.232	0.033	0.231
100	0.065	0.265	0.0451	0.232	0.0217	0.231
150	0.0669	0.266	0.0431	0.232	0.0162	0.231
200	0.068	0.266	0.0417	0.232	0.0129	0.231
250	0.0687	0.266	0.0406	0.231	0.0108	0.231

Table 5: Summary of modeled maximum depth of chemical in the soil column and days to maximum ()**[SoilMaxDepth]**

Annual Rainfall (inches)	Clay		Loam		Sand	
	Depth	Julian Day	Depth	Julian Day	Depth	Julian Day
5	6.5	1991180	6.5	1991180	6.5	1991180
10	6.5	1991180	6.5	1991180	6.5	1991180
15	12	1991181	18	1991181	30	1991221
20	12	1991181	18	1991181	42	1992031
25	18	1991212	24	1991191	48	1992001
50	18	1991191	36	1992001	60	1991231
100	18	1991182	42	1991261	60	1991181
150	18	1991181	54	1993041	60	1991181
200	18	1991181	60	1992101	60	1991181
250	18	1991181	60	1991361	60	1991181

Table 6: Summary of the cumulative loss from soil runoff and sediment as a proportion of the application rate **[PropRunoSed]**

Annual Rainfall (inches)	Clay	Loam	Sand
5	0	0	0
10	0	0	0
15	0	0	0
20	0	0	0
25	0	0	0
50	0	2.29E-07	0
100	0	9.23E-06	0
150	0	1.21E-06	0
200	0	1.78E-08	0
250	0	1.72E-08	0