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Spinosad:
Human Health and Ecological Risk Assessment
FINAL REPORT

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
AEL	adverse-effect level
a.e.	acid equivalent
a.i.	active ingredient
a.k.a.	also known as
a.s.	active substance
ATPase	adenylpyrophosphatase
APHIS	Animal and Plant Health Inspection Service
ATSDR	Agency for Toxic Substances and Disease Registry
ASAE	American Society of Agricultural Engineers
BCF	bioconcentration factor
bw	body weight
calc	calculated value
CBI	confidential business information
CI	confidence interval
cm	centimeter
CNS	central nervous system
COC	crop oil concentrates
DAA	days after application
DAT	days after treatment
DER	data evaluation record
d.f.	degrees of freedom
DG	dispersible granule
EC	emulsifiable concentrate
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
ECOTOX	ECOTOXicology (database used by U.S. EPA/OPP)
EFED	Environmental Fate and Effects Division (U.S. EPA/OPP)
ExToxNet	Extension Toxicology Network
F	female
FH	Forest Health
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FIRST	FQPA Index Reservoir Screening Tool
FOB	Functional Observational Battery (behavioral assays)
FQPA	Food Quality Protection Act
g	gram
GABA	gamma-aminobutyric acid
GLP	Good Laboratory Practices
ha	hectare
HED	Health Effects Division (U.S. EPA/OPP)
HQ	hazard quotient
HRAC	Herbicide Resistance Action Committee
IARC	International Agency for Research on Cancer
IRED	Interim Reregistration Eligibility Decision

IRIS	Integrated Risk Information System
k_a	absorption coefficient
k_e	elimination coefficient
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
$K_{o/w}$	octanol-water partition coefficient
K_p	skin permeability coefficient
L	liter
lb	pound
LC_{50}	lethal concentration, 50% kill
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level
LOC	level of concern
LR_{50}	50% lethal response [EFSA/European term]
m	meter
M	male
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
mM	millimole
mPa	millipascal, (0.001 Pa)
MOS	margin of safety
MRID	Master Record Identification Number
MSDS	material safety data sheet
MSO	methylated seed oil
MW	molecular weight
nAChR	nicotinic acetylcholine receptor
NAWQA	USGS National Water Quality Assessment
NCI	National Cancer Institute
NCOD	National Drinking Water Contaminant Occurrence Database
NIOSH	National Institute for Occupational Safety and Health
NIS	nonionic surfactant
NOAA	National Oceanic and Atmospheric Administration
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOS	not otherwise specified
N.R.	not reported
OM	organic matter
OPP	Office of Pesticide Programs
OPPTS	Office of Pesticide Planning and Toxic Substances
OSHA	Occupational Safety and Health Administration
Pa	Pascal
PBPK	physiologically-based kinetic
PRZM-GW	Pesticide Root Zone Model for Ground Water
ppm	parts per million
RBC	red blood cells

RED	re-registration eligibility decision
RfD	reference dose
SC	Suspension concentrate
SDS	Safety Data Sheet
SERA	Syracuse Environmental Research Associates
SRBC	sheep red blood cells
TEP	typical end-use product
T.G.I.A.	Technical grade active ingredient
TRED	Tolerance Reassessment Eligibility Decision
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
VMD	volume median diameter (for droplet size distributions)
WHO	World Health Organization
WWSA	Weed Science Society of America

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℃+32
centimeters	inches	0.3937
cubic meters (m^3)	liters (L)	1,000
Fahrenheit	centigrade	0.556℉-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

Spinosad is the common name for a natural insecticide that is formed in fermentation by the *Saccharopolyspora spinosa* (Actinobacteria: Actinomycetales). Spinosad is cited as a *biorational pesticide* in the open literature and is classified as a reduced risk pesticide by the EPA. Spinosad is used to control numerous insect populations (e.g., lepidopteran larvae, flies, thrips and beetles) on various agricultural crops and nonagricultural sites, including tree farms. The Forest Service evaluated the use of spinosad to control coneworms (*Dioryctria* species) and seed bugs (*Leptoglossus corculus*) in loblolly pine seed orchards. In addition, the Forest Service is considering the use of spinosad to control minor infestations of pine sawflies and other defoliators in and around recreation areas, district offices, work centers, and other areas where conventional agricultural pesticides would not be appropriate.

Spinosad formulations labelled for forestry may be applied by directed foliar, ground broadcast foliar, or aerial foliar applications. All three of these application methods are explicitly covered in the current risk assessment. The risk assessment also explicitly considers a single application at a rate of 0.225 lb a.i./acre and two applications at the same rate with a 6-day application interval. This two-application scenario equals the maximum seasonal application rate of 0.45 lb a.i./acre using the minimum application interval for trees specified on the product labels.

In both the human health and ecological risk assessments, the quantitative expression of the risk characterization is the hazard quotient (HQ), the ratio of the anticipated dose or exposure to the RfD (human health) or no-observed-effect level or concentration (ecological effects) using 1 as the level of concern—i.e., an HQ of < 1 is below the level of concern.

Potential risks to humans are minimal to marginal for most exposure scenarios. Upper bound HQs associated with the consumption of contaminated vegetation are a concern but the associated exposure scenarios should be viewed as extreme. The nontarget organisms at greatest risk are the invertebrates, both terrestrial and aquatic. Analogous to the human health risk assessment, risks to vertebrate wildlife are much lower than potential risks to invertebrates.

Consistent with the EPA occupational risk assessments, none of the estimates for general exposures of workers developed in the current risk assessment result in HQs that exceed the level of concern (HQ=1) even at the upper bounds. Similarly, none of the accidental exposure scenarios for workers approach a level of concern. A residual concern for workers involves the potential for eye irritation. The studies reviewed by EPA do not suggest that spinosad is likely to be an eye irritant, and none of the product labels requires eye protection; on the other hand, the MSDS/SDS for some formulations suggest the potential for moderate to serious eye irritation, and all of the MSDS/SDS recommend the use of protective eyewear. Hence, the use of protective eyewear would be prudent in any application of these formulations.

The only non-accidental exposure scenarios for members of the general public that exceed the level of concern involve the consumption of contaminated vegetation (following a single application or two applications) and the consumption of contaminated fruit (following two applications). The HQs that exceed the level of concern range from 1.1 to 12. Based on dose-severity relationships, the HQ of 1.1 (the central estimate of exposure for the consumption of

contaminated vegetation following two applications) does not raise substantial concern. While the upper bound HQs associated with contaminated vegetation or fruit (i.e., HQs from 1.6-12) would probably not be associated with frank signs of toxicity, the levels of exposure exceed what would be considered acceptable. If spinosad is sprayed on vegetation that might be consumed by humans, measures should be taken to mitigate exposures to members of the general public.

HQs associated with accidental exposure scenarios for members of the general public do not exceed the level of concern for direct spray; nevertheless, some HQs for the accidental spill scenarios do exceed the level of concern with a maximum HQ of 15 (i.e., the consumption of contaminated fish by subsistence populations). While there is no direct evidence that these scenarios would result in observable signs of toxicity, these HQs justify measures to reduce/mitigate exposures to members of the general public.

In terms of ecological risks, adverse effects are virtually certain in sensitive species of phytophagous insects. Spinosad will be applied to terrestrial vegetation. Sensitive species of phytophagous insects that consume the contaminated vegetation will likely be killed. This risk characterization pertains to virtually any insecticide applied to vegetation at an effective application rate.

Potential risks to bees are also apparent but vary depending on the route of exposure. Honeybees as well as other insects that are directly sprayed with spinosad will probably be killed. Based on a single study, *Bombus terrestris*, a species of bumblebee, appears to be less sensitive than honeybees in terms of contact exposures. In the absence of a replicate and confirming study, bumblebees are considered a group at potential risk following direct spray. Foliar interception of spinosad residues will substantially reduce risks to terrestrial insects. As a mitigating factor in risks to bees, the product labels for all formulations of spinosad indicate that the product should not be applied while bees are actively foraging. This limitation will substantially reduce risks to honeybees associated with direct spray or spray drift. The impact of these limitations on risks associated with foraging are less clear.

The HQs for foraging honeybees exposed to contaminated nectar are less than the HQs associated with direct spray; nonetheless, risks to foraging honeybees are substantial based on dose estimates associated with foraging for contaminated nectar. While there are substantial uncertainties with the exposure assessment presented in the current risk assessment, these uncertainties do not negate concerns for potential effects on honeybees and other pollinators via contaminated nectar following applications of spinosad. Most field or field simulation studies on risks to honeybees are not published in the open literature. Nonetheless, reasonably detailed reviews of these studies are available, and these field and field simulation studies do not indicate significant or substantial risks to foraging bees at application rates considered by the Forest Service. The available field studies are limited in that the studies are relatively short-term and focused on spray exposures rather than foraging. A field simulation study conducted over exposure periods of 3 to 5 weeks does raise concern for decreases in foraging activity at an exposure equivalent to an application rate of about 0.07 lb a.i./acre. Longer-term field studies on colony health, including observations on colony overwintering, are not available.

Aquatic invertebrates, particularly sensitive species, could be at substantial risk following the application of spinosad in areas where the potential for water contamination is high, including areas with moderate to heavy rainfall. In arid areas, particularly areas with predominantly loam or sand soil textures, adverse effects on even sensitive species of aquatic invertebrates might not be observed. Given the variability in the estimated concentrations of spinosad in water, no general risk characterization for aquatic invertebrates is justified. In any site-specific application of spinosad, the risks will vary substantially with local conditions. Given the highly variable results from the generic water modeling used in the current risk assessment and the substantial impact that this variability has on the risk characterization for aquatic invertebrates, site-specific efforts to estimate surface water concentrations of spinosad might be justified, particularly in areas with moderate to heavy rainfall.

Vertebrates are less sensitive than invertebrates to spinosad. Nonetheless, foliar applications of spinosad could result in exposure levels that exceed the level of concern for some terrestrial mammals (longer-term exposures only) and birds (both acute and longer-term). For non-accidental exposure scenarios, risks to mammals and birds are associated with the consumption of contaminated vegetation, and risks are greatest for smaller animals consuming contaminated grasses or food items with spinosad concentrations comparable to those associated with contaminated grasses. The only HQ for accidental exposure scenario for terrestrial vertebrates that exceeds the level of concern is the upper bound HQ for a canid consuming contaminated fish. Except for an accidental spill scenario, risks to fish and aquatic vegetation appear to be insubstantial.

The risk characterization for spinosad focuses on the potential for direct toxic effects. Nonetheless, there is a potential for secondary or indirect effects in virtually all groups of nontarget organisms. Terrestrial applications of any effective insecticide, including spinosad, are likely to alter insect and other invertebrate populations within the treatment area. This alteration could have indirect effects on terrestrial or aquatic animals and plants, including changes in food availability, predation, and habitat quality. These indirect effects may be beneficial to some species and detrimental to others; moreover, the magnitude of indirect effects is likely to vary over time.

1. INTRODUCTION

1.1. Chemical Specific Information

This document provides human health and ecological risk assessments addressing the consequences of spinosad use in Forest Service programs to control insect pests. As discussed in Section 2.2, spinosad is an insecticide that controls a broad spectrum of insects that can damage vegetation. The USDA/Forest Service evaluated the use of spinosad to control insect pests (e.g., Nowak et al. 2000, 2001, 2010) but has not developed a full risk assessment until now. The USDA's Animal and Plant health protection service has developed both human health and ecological risk assessments on spinosad (USDA/APHIS 1999, 2003, 2011, 2014).

Initially, the published literature on spinosad was identified using TOXLINE (http://toxnet.nlm.nih.gov/) and ECOTOX (http://cfpub.epa.gov/ecotox/). Additional information on spinosad was identified through standard Internet search engines and databases (e.g., HSDB 2010; Kegley et al. 2014). As summarized in Table 1, the open literature on spinosad is substantial. As with many insecticides, most of the published studies on spinosad involve assays or field applications focused on evaluating efficacy on various crops and against a variety of target terrestrial insects. As with all Forest Service risk assessments on insecticides, efficacy studies are not covered extensively; nevertheless, some of these studies, particularly those involving the assessment of resistance, are used to define differences in sensitivity between target and nontarget insects as well as variability in sensitivity among different populations of terrestrial insects. Numerous studies are available on nontarget insects, including bees, and this literature is covered in some detail. The literature on aquatic species is focused on aquatic invertebrates including species of mosquito larvae, which are target species (e.g., Kirst et al. 1992; Perez et al. 2007). Open literature on vertebrates, particularly fish, is sparse. Spinosad has been used medicinally in humans and domestic/agricultural mammals to treat lice and other pest insects. The veterinary literature provides some information on the toxicity of spinosad to species such as dogs, cats, and sheep. Only one study has been identified on a case of human poisoning (i.e., Su et al. 2011). The open literature on the environmental fate of spinosad is modest; nonetheless, several studies on the fate of spinosad in plants are directly useful in the exposure assessments for humans and other terrestrial species. A modest literature is available on forestry applications of spinosad, as discussed further in Section 2 (Program Description).

In addition to the open literature on spinosad, the available studies conducted by or for registrants of spinosad constitute much of the data most relevant to the assessment of potential risks to humans and the environment. The U.S. EPA's Office of Pesticide Programs (U.S. EPA/OPP) has the regulatory authority for the registration of pesticides. As discussed in Section 2.2, spinosad was registered originally in the United States in 1997. For many pesticides, studies required for registration and reregistration are summarized in a Reregistration Eligibility Decision (RED) document. Because spinosad was registered in 1997 (i.e., relatively recently), it was not subject to the reregistration process under FIFRA (U.S. EPA/OPP 2012a, p. 4). Nonetheless, several EPA risk assessments on spinosad are available, including risk assessments focused on human health effects (i.e., U.S. EPA/OPP/HED 1997a,b, 2007a, 2009a, 2009b, 2010a,b, 2011a) and ecological effects (U.S. EPA/OPP/EFED 2005, 2009a, 2010a, 2011a). In addition, an EPA web site (http://iaspub.epa.gov/apex/pesticides/f?p=CHEMICALSEARCH:3:0::NO:1,3,31,7,12,25:P3_X

CHEMICAL_ID:3922) contains summaries of registrant studies in the form of Data Evaluation Records (DERs). As discussed further in Section 1.2, the risk assessments and related documents from U.S. EPA/OPP include summaries of the required registrant studies submitted to the EPA. The registrant submitted studies are not available to the general public and were not available during the conduct of the current risk assessment. Nonetheless, relevant information on these registrant-submitted studies is available in the EPA risk assessments cited above. Registrant-submitted studies are designated by EPA using Master Record Identification Numbers (MRID numbers). In the appendices to and text of the current risk assessment, the registrant studies are identified by MRID number and the source of the information—i.e., the specific risk assessment from EPA—is specified for each of the studies summarized in the appendices. Summaries based on DERs are designated in standard author(s)/date format along with the MRID number – e.g., Albee et al. 1994/MRID 43557501.

The U.S. EPA has developed a registration review program for pesticides which operates on a 15-year cycle. Spinosad is currently under registration review which is scheduled for completion in 2017 (U.S. EPA/OPP 2012a, p. 8). While the final risk assessments on spinosad from the registration review will not be available during the conduct of the current Forest Service risk assessment, several relevant documents in support of the registration review have been released by EPA and are used in the preparation of the current risk assessment (U.S. EPA/OPP 2011b,2012a; U.S. EPA/OPP/HED 2011a (registration review scoping), U.S. EPA/OPP/HED 2011b (human incidents); U.S. EPA/OPP/HED 2012a (response to public comment); U.S. EPA/OPP/EFED 2011a (preliminary assessment) U.S. EPA/OPP/EFED 2012a (response to public comments); U.S. EPA/OPP/EFED 2009b; U.S. EPA/OPP/EFED 2011a).

In addition to the documents from EPA, additional risk assessments are available from USDA's Animal and Plant health protection service (USDA/APHIS 1999, 2003, 2011, 2014) as cited above, the European Food Safety Authority (EFSA 2011, 2012, 2013, 2014), the World Health Organization (FAO/WHO 2001; WHO 2008, 2011), and reviews and assessments in the open literature (Biondi et al. 2012; Cleveland et al. 2002a,b; Dow 2014; Dow Elanco 1996; Elanco 2012; Gao et al. 2007b; HSDB 2003 [Spinosyn-A only]; Kirst et al. 1992; Mandal et al. 2013; Mayes et al. 2003; McCormack 2011; McFadden and Saunders 2004; Miles and Eelen 2006; Sparks et al. 1998; Thompson et al. 2015; Williams et al. 2003b). For the most part, reviews of spinosad are used primarily to identify key studies from the open literature and not as direct sources of information. Exceptions to this approach are discussed in the body of this risk assessment as appropriate.

1.2. General Information

This document has four narrative sections, including the introduction (Section 1), program description (Section 2), risk assessment for human health effects (Section 3), and risk assessment for ecological effects or effects on wildlife species (Section 4). Each of the two risk assessment sections has four major subsections, including an identification of the hazards, an assessment of potential exposure, an assessment of the dose-response relationships, and a characterization of the risks associated with plausible levels of exposure.

This is a technical support document which addresses some specialized technical areas. Nevertheless an effort was made to ensure that the document can be understood by individuals who do not have specialized training in the chemical and biological sciences. Certain technical

concepts, methods, and terms common to all parts of the risk assessment are described in plain language in a separate document (SERA 2014a). The human health and ecological risk assessments presented in this document are not intended to be comprehensive summaries of all of the available information. On the other hand, the information in the appendices as well as the discussions in Sections 2, 3, and 4 of the risk assessment are intended to be detailed enough to support an independent review of the risk analyses.

As noted in Section 1.1, the studies submitted in support of the registration of spinosad are used extensively in this risk assessment based on information publically available from the U.S. EPA. In any risk assessment based substantially on registrant-submitted studies, the Forest Service is sensitive to concerns from members of the general public of potential bias. The general concern might be expressed as follows:

> *If the study is paid for and/or conducted by the registrant, the study may be designed and/or conducted and/or reported in a manner that will obscure any adverse effects that the compound may have.*

This concern is largely without foundation. While any study (published or unpublished) can be falsified, concerns with the design, conduct and reporting of studies submitted to the U.S. EPA for pesticide registration are minor. The design of the studies submitted for pesticide registration is based on strict guidelines for both the conduct and reporting of studies. These guidelines are developed by the U.S. EPA and not by the registrants. Full copies of the guidelines for these studies are available at http://www2.epa.gov/test-guidelines-pesticides-and-toxic-substances. Virtually all studies accepted by the U.S. EPA/OPP are conducted under Good Laboratory Practices (GLPs). GLPs are an elaborate set of procedures which involve documentation and independent quality control and quality assurance that substantially exceed the levels typically seen in open literature publications. As a final point, the EPA reviews each submitted study for adherence to the relevant study guidelines. These reviews most often take the form of Data Evaluation Records (DERs). While the nature and complexity of DERs varies according to the nature and complexity of the particular studies, each DER involves an independent assessment of the study to ensure that the EPA Guidelines are followed and that the results are expressed accurately. In many instances, the U.S. EPA/OPP will reanalyze raw data from the study as a check or elaboration of data analyses presented in the study. In addition, each DER undergoes internal review (and sometimes several layers of review). The DERs prepared by the U.S. EPA form the basis of EPA risk assessments and, when available, DERs are used in Forest Service risk assessments. The specific DERs used in the current Forest Service risk assessment are identified in Section 5 (References) as DER01.

While data quality and data integrity are not substantial concerns, a limitation in risk assessments based substantially on registrant-submitted studies involves the nature and diversity of the available studies. The studies required by the U.S. EPA are based on a relatively narrow set of criteria in a relatively small subset of species and follow standardized protocols. The relevance of this limitation to the current risk assessment on spinosad is noted in various parts of this risk assessment as appropriate. As discussed in Section 1.1, the open literature on spinosad is focused on efficacy studies but includes studies relevant to the assessment of both potential human health effects as well as effects on terrestrial and aquatic nontarget species. The open

literature is used quantitatively in the current risk assessment as appropriate. Any use of open literature data in preference to registrant studies used by the EPA is discussed in detail in the body of this risk assessment.

The Forest Service periodically updates pesticide risk assessments and welcomes input from the general public and other interested parties on the selection of studies included in risk assessments. 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.

As with all Forest Service 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 quite 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 which are relatively simple. Simple calculations are included in the body of the document [typically in brackets]. The results of some calculations within brackets may contain an inordinate number of significant figures in the interest of transparency—i.e., to allow readers to reproduce and check the calculations. In all cases, these numbers are not used directly but are rounded to the number of significant figures (typically two or three) that can be justified by the data.

Notwithstanding the above, some of the calculations used in this risk assessment are cumbersome. For those calculations, EXCEL workbooks (i.e., sets of EXCEL worksheets) are included as attachments to this risk assessment. The workbooks included with the current risk assessment are discussed in Section 2.4. The worksheets in these workbooks provide the detail for the estimates cited in the body of the document. Documentation for the use of these workbooks is presented in SERA (2011a).

The EXCEL workbooks are integral parts of the risk assessment. The worksheets contained in these workbooks are designed to isolate the numerous calculations from the risk assessment narrative. In general, all calculations of exposure scenarios and quantitative risk characterizations are derived and contained in the worksheets.

In the EXCEL worksheets as well as in the text of this risk assessment, the hazard quotient (HQ) is used to characterize risk. The HQ is the ratio of the estimated exposure to a toxicity value, typically a no adverse effect level or concentration (e.g. RfD, NOAEL or NOAEC). Both the rationale for the calculations and the interpretation of the hazard quotients are contained in this risk assessment document. A fuller discussion of the use of HQs is included in SERA (2014a).

2. PROGRAMS DESCRIPTION

2.1. Overview

Spinosad is the common name for a natural insecticide that is formed in fermentation by the *Saccharopolyspora spinosa* (Actinobacteria: Actinomycetales). Spinosad is a mixture of two similar components, spinosyn A (the major component) and spinosyn D (the minor component). Spinosad is cited as a *biorational pesticide* in the open literature and is classified as a reduced risk pesticide by the EPA. While the components of spinosad degrade relatively rapidly in the environment, the degradation products are similar to the parent compounds. Accordingly, the EPA views spinosad as functionally persistent in the environment, given that degradates of the spinosyns are so similar in toxicity to the parent compounds.

Spinosad is a broad spectrum pesticide registered for the control of numerous insects (e.g., lepidopteran larvae, flies, thrips and beetles) on various agricultural crops and nonagricultural sites, including tree farms. Spinosad has been evaluated by the Forest Service for the control of coneworms (*Dioryctria* species) and seed bugs (*Leptoglossus corculus*) in loblolly pine seed orchards. In addition, the Forest Service is considering the use of spinosad to control minor infestations of pine sawflies and other defoliators in and around recreation areas, district offices, work centers and other areas where conventional agricultural pesticides would not be appropriate.

Based on the open literature involving forestry applications, representative formulations included explicitly in the current risk assessment consist of a dispersible granule (Blackhawk), a wettable powder (Entrust), and three suspension concentrates (Conserve SC, Entrust SC, and SpinTor 2SC). These and other formulations of spinosad labelled for forestry may be applied by directed foliar, ground broadcast foliar, or aerial foliar applications. All three of these application methods are explicitly covered in the current risk assessment. The risk assessment also explicitly considers a single application at a rate of 0.225 lb a.i./acre and two applications at the same rate with a 6-day application interval. This two-application scenario equals the maximum seasonal application rate of 0.45 lb a.i./acre using the minimum application interval for trees specified on the product labels.

Spinosad is closely related to spinetoram, a newer pesticide consisting of spinosyns J and L which are structurally related to but not identical to spinosyns A and D. The current Forest Service risk assessment is concerned primarily with spinosad; hence, information on spinetoram is not considered except as necessary to discuss EPA toxicity values for spinosad. Based on use statistics from both USGS and the state of California, spinetoram appears to be displacing spinosad, at least in agricultural applications. Because spinosad has not been used extensively in Forest Service programs or projects, it is unclear at this time if Forest Service applications of spinosad would be negligible, relative to agricultural applications of this pesticide.

2.2. Chemical Description and Commercial Formulations

As illustrated in Figure 1, both spinosyn A and spinosyn D are structurally complex, consisting of a tetracyclic macrolide ring system (i.e., a macrocyclic lactone ring with 12 or more elements), forosamine and rhamnose sugars, and methyl groups. Alternate designations are sometimes used for spinosyns A and D—e.g., factor A and factor D in several EPA risk assessments. The somewhat more specific designations of spinosyn A and spinosyn D used by

WHO (2011) are used consistently in the current risk assessment. While both IUPAC and CAS names are available for spinosyn A and D, the names are long, cumbersome, and not used in the current risk assessment. As also illustrated in Figure 1, spinosyn A and spinosyn D differ only in the presence of a methyl group on 4-carbon of the macrolide ring.

The chemical and physical properties of spinosad are summarized in Table 2. Spinosad, particularly spinosyn A, has a high affinity for soils with most K_{oc} values greater than 1000. While both spinosyns A and D are highly lipophilic (i.e., high K_{ow} values), these compounds do not tend to bioconcentrate substantially in fish (BCFs below 100). Both spinosyn A and spinosyn D have relatively short half-lives in soil (<20 days) but are metabolized in the environment to compounds that are very similar to the parent compounds (U.S. EPA/OPP/EFED 2011a). Minor metabolic pathways (i.e., demethylation) have also been noted in mammals (FAO/WHO 2011). Consequently, as discussed further in Section 3.2.3.4 (Contaminated Water), the modeled water concentrations of spinosad assume that spinosad is essentially stable, which is identical to the approach used by EPA in drinking water assessments (e.g., U.S. EPA/OPP/EFED 2009b).

The ratio of spinosyn A to spinosyn D in technical grade spinosad appears to be highly variable. The U.S. EPA (e.g., U.S. EPA/OPP/EFED 2011a, p. 11) and WHO (2008) indicate that the ratio of spinosyns A:D may vary from 50:50 to 95:5 and that a typical ratio is 85:15 (i.e., equivalent to 17:3 or about 5.7:1). The variability in the ratios of spinosyns A:D does not appear to be a significant source of uncertainty in the current risk assessment. As discussed further in Section 3.1.5, subchronic bioassays of spinosad with spinosyn A:D ratios of 1:1 and 5:1 appear to have similar toxicities in mammals. Similar studies on receptors of interest to the ecological risk assessment, however, have not been identified.

Spinosad is closely related to spinetoram, a chemically modified mixture of spinosyns J and L (Dow 2014b). Several EPA human health risk assessments jointly consider spinosyn and spinetoram (e.g., U.S. EPA/OPP 2009a, U.S. EPA/OPP 2011a). Human health risk assessments conducted by the EPA typically consider spinosad and spinetoram as toxicologically equivalent (e.g., U.S. EPA/OPP/HED 2009a, 2011a). As discussed further in Section 3.4, the chronic RfD for spinosad is based on a study with spinetoram. As discussed in Section 4.1, this toxicological equivalence does not hold for the ecological risk assessment, and spinosad is more toxic to terrestrial invertebrates but less toxic to aquatic invertebrates than spinetoram (e.g., U.S. EPA/OPP 2012a, p. 4). The current Forest Service risk assessment is concerned primarily with spinosad. Data on spinetoram are not considered except as necessary to discuss toxicity values used in the current Forest Service risk assessment and EPA risk assessments.

Spinosad is often referenced in the literature as a "biorational" pesticide (e.g., Jiang and Mulla 2009; Marina et al. 2012; Nowak et al. 2001). The term *biorational pesticide* is generally used to designate pesticides that involve low application rates and few nontarget effects (Hall and Barry 1995; Horowitz et al. 2009). Consistent with the use of this term in the open literature, the U.S. EPA/OPP (2015a) designates spinosad as a "reduced risk" pesticide—i.e., a pesticide that generally poses fewer risks to humans and other nontarget organisms relative to conventional pesticides.

The spinosyns are related structurally to a large class of drugs with a macrocyclic lactone ring. These compounds are used as antibiotics, antifungals, drugs that promote gastric emptying, and immunosuppressants (e.g., Kanoh and Rubin 2010). The structural similarity, however, does not appear to hold in terms of pharmacology. The macrolide drugs appear to inhibit neurotransmitter release, but neurotoxicity is not a primary mode of action (Kanoh and Rubin 2010).

While spinosad was discovered in the early 1980s (Thompson et al. 2000; Tomlin 2004), it was not registered as a pesticide in the United States until 1997 (U.S. EPA/OPP 2012a). Spinosad was originally registered by DowElanco (now Dow AgroSciences) (Thompson 2015; Tomlin 2004; U.S. EPA/OPP/HED 1997) but appears to be off patent with 76 active formulations from several different companies available in the United States (Kegley et al. 2014).

Spinosad is a broad spectrum pesticide registered for the control of many insect populations (e.g., lepidopteran larvae, flies, thrips and beetles) on various agricultural crops and nonagricultural sites, including tree farms (Dow 2014a; Harrell and Stepanek 2005; Semiz et al. 2006; U.S. EPA/OPP 2012a; Wanner et al. 2002). The U.S. EPA granted an emergency exemption to the state of Michigan on June 18, 2010 for the control of Emerald Ash Borer on wood lots (http://iaspub.epa.gov/apex/pesticides/f?p=CHEMICALSEARCH:12:0::NO:1,3,31,7,12,25:P3_XCHEMICAL_ID:3922) and this use has been evaluated by the Forest Service (Lewis et al. 2007). Some formulations of spinosad (e.g., Conserve SC and Entrust) are now specifically labelled for the control of the emerald ash borer. These and other formulations of spinosad labelled for forestry use are discussed further below.

The Forest Service has indicated that spinosad may be used for the control of coneworms (*Dioryctria* species) and seed bugs (*Leptoglossus corculus*) in a loblolly pine seed orchards (Mangini 2016) as well as the Nantucket pine tip moth (Nowak et al. 2010). In addition, the Forest Service is considering the use of spinosad to control minor infestations of pine sawflies and other defoliators in and around recreation areas, district offices, work centers and other areas where conventional agricultural pesticides would not be appropriate.

Representative formulations of spinosad labelled for forestry are given in Table 3. The representative formulations include a dispersible granule (Blackhawk), a wettable powder (Entrust), and three suspension concentrates (Conserve SC, Entrust SC, and SpinTor 2SC). Forestry applications of Conserve are documented in the literature for the control of the Douglas-fir tussock moth, *Orgyia pseudotsugata* (Cranshaw et al. 2014) and several other insect pests on conifers (Nebraska Forest Service 2009). The use of Spin Tor 2SC is documented in the literature for the control of the Nantucket pine tip moth, *Rhyaciona frustrana* (Nowak et al. 2000). Spinosad is also used to protect fruit orchards from various insect pests (e.g., Peusens and Belian 2012).

The list of formulations in Table 3 is not intended to be exclusive. Other formulations of spinosad are available commercially and new formulations of spinosad may become available in the future. The Forest Service may elect to use registered formulations of spinosad relevant to forestry applications other than those summarized in Table 3. If other formulations are used in Forest Service programs, however, attempts should be made to identify information on the inerts

in the formulations as well as the toxicity of the formulations to ensure that the formulation under consideration is comparable to the formulations explicitly designated in Table 3.

Some information on mammalian toxicity as well as toxicity to nontarget organisms is typically given on MSDSs or SDSs for the formulations. Information on mammalian toxicity from the MSDSs/SDSs is summarized in Table 4. Note that the six types of studies summarized in Table 4 – i.e., acute oral, dermal, and inhalation as well as dermal irritation, eye irritation, and skin sensitization -- are sometimes referred to as the "mammalian six-pack". These types of studies are typically required by the EPA on all unique formulations (NAS 2013). Information on ecological effects from the MSDSs/SDSs is summarized in Table 5. These types of studies are typically required by EPA on the active ingredient but some of these studies may be conducted on formulations.

If information on mammalian and ecological receptor toxicity from MSDSs/SDSs for another formulation is comparable to the information given in Tables 4 and 5, the other formulation would be encompassed by the current risk assessment unless additional information (e.g., new literature or case reports on the formulation) suggest that the other formulation may be more hazardous than the representative formulations specified in Table 3. The data in Tables 4 and 5 are discussed in subsequent sections of this risk assessment as appropriate.

2.3. Application Methods

All formulations of spinosad listed in Table 3 are labelled for both ground applications (directed and broadcast foliar) and aerial broadcast foliar applications. Since the Forest Service generally avoids aerial applications, ground applications are most commonly used in Forest Service programs. Since the Forest Service has conducted an aerial application of spinosad to control the emerald ash borer (Lewis et al. 2007), aerial applications of spinosad are considered explicitly in the current risk assessment. Other forestry applications of spinosad involved backpack applications (Nowak et al. 2000); hence, backpack applications and ground broadcast applications are considered explicitly in the current risk assessment.

As discussed in Section 1.1, this risk assessment is accompanied by EXCEL workbooks that detail the exposure scenarios for spinosad. Based on the anticipated uses of spinosad in Forest Service programs, two EXCEL workbooks are provided, one for a single application (Attachment 1) and the other for multiple applications of spinosad (Attachment 2). The specific application rates and intervals are discussed further in the following section.

Different application methods involve different estimates of the amount of a pesticide used by workers in a single day based on the number of acres treated per day and the application rate. Application rates are discussed in Section 2.4, and assumptions about the number of acres treated by a worker in a single day are discussed further in Section 3.2.2 (worker exposure assessments).

2.4. Mixing and Application Rates

As discussed in EPA's assessment for the registration review of spinosad, maximum single application rates vary substantially by crop ranging from about 0.0003 to 0.765 lb a.i./acre (U.S. EPA/OPP/EFED 2011a, p. 10). The upper range of 0.765 lb a.i./acre applies specifically to woody plants. Much lower application rates, however, are noted in published forestry applications of spinosad—i.e., about 0.087 lb a.i./acre [0.098 kg a.i./ha] in the publication by

Nowak et al. (2000) and 0.225 lb a.i./acre [7.2 oz formulation/acre ÷ 128 oz/gallon x 4 lb a.i./gallon formulation] in the publication by Lewis et al. (2009).

As summarized in Table 3, the maximum seasonal application rate for the representative forestry formulations of spinosad is 0.45 lb a.i./acre. Oddly, the product label for Conserve SC seems somewhat ambiguous. Like other labels for forestry formulations, the product label for Conserve SC indicates that the maximum seasonal application rate is 0.45 lb a.i./acre; however, it also indicates that up to 88 fluid ounces of the formulation may be applied per acre for the control of some tree pests such as the emerald ash borer. For this 1 lb a.i./gallon formulation, 88 fluid ounces/acre corresponds to an application rate of about 0.69 lb a.i./acre, which exceeds the maximum labelled seasonal application rate of 0.45 lb a.i./acre which appears to apply to forestry applications. For some non-forestry applications, the most recent human health risk assessment from EPA does consider application rates of up to about 0.76 lb a.i./acre (U.S. EPA/OPP/HED 2011a, p. 48).

For the current Forest Service risk assessment, the maximum single application rate (detailed in Attachment 1) is taken as 0.225 lb a.i./acre from the forestry application by Lewis et al. (2009). This study was a joint effort by APHIS, the University of Michigan, and the USDA Forest Service. For multiple applications (Attachment 2), two applications of 0.225 lb a.i./acre with an application interval of 6 days is used. This two-application scenario equals the maximum seasonal application rate of 0.45 lb a.i./acre using the minimum application interval for trees specified on the forestry labels. The maximum seasonal application rate of 0.45 lb a.i./acre is consistent with recent risk assessments from EPA (U.S. EPA/OPP/HED 2009a, p. 13).

Application volumes, meaning the number of gallons of pesticide solution applied per acre, have an impact on the estimates of potential risk. The extent to which a formulation is diluted prior to application primarily influences dermal and direct spray exposure scenarios, both of which depend on 'field dilution' (i.e., the concentration of spinosad in the applied spray). In all cases, higher herbicide concentrations (i.e., equivalent to the lower dilution of the herbicide) increase the estimate of exposure and hence risk. As summarized in Table 3, minimum application volumes of 5 to 10 gallons per acre are recommended. In the workbooks that accompany this risk assessment, the application volumes are taken as 10 (5 to 20) gallons per acre.

The selection of specific application rates and dilution volumes in this risk assessment is intended to reflect plausible estimates of potential exposures. In the assessment of specific program activities, the application rates and volumes can be changed in Worksheet A01 of the EXCEL workbooks to reflect the rates and volumes that are actually used in any specific application of spinosad.

2.5. Use Statistics

Forest Service risk assessments attempt to characterize the use of an herbicide or other pesticide in Forest Service programs relative to the use of the herbicide or other pesticide in agricultural applications. Forest Service pesticide use reports up to the year 2004 are available on the Forest Service web site (http://www.fs.fed.us/ foresthealth/pesticide/reports.shtml). No applications of spinosad are noted in these reports.

Information on the agricultural use of pesticides is compiled by the U.S. Geological Survey (USGS) (http://water.usgs.gov/nawqa/pnsp/usage/maps/). This web site does not contain an entry for "spinosad" but does contain entries labelled "Spinosyn" and "Spinetoram." While somewhat speculative, it appears that the entry for "Spinosyn" represents agricultural applications of spinosad because "spinosyn" is not a registered pesticide and spinosad is the only registered pesticide other than spinetoram that consists of spinosyns. Under this assumption, the agricultural use of spinosad in 2012, the most recent year for which data are available, is estimated by the USGS (2015) to range from about 50,000 lbs (Figure 2) to 60,000 lbs (Figure 3). The greatest use of spinosad is in the south central United States, encompassed by Forest Service Region 8, with additional concentrations of use in California (Forest Service Region 5) and the Pacific Northwest (Forest Service Region 6). Based on use data by crop (also summarized in Figure 2 and Figure 3), spinosad is currently used primarily on vegetables and fruit including grapes and orchards. The temporal pattern in the use of spinosad is noteworthy with a substantial decrease in use from a maximum of about 0.24 million pounds in 2002 to about 0.05 million pounds in 2012. This decrease in use of "spinosyn"/spinosad is accompanied by a sharp increase in the agricultural use of spinetoram from 2008 to 2012. As noted by U.S. EPA/OPP (2011a, p. 4), *Dow anticipates that the use of spinetoram will continue to increase and displace spinosad uses due to its enhanced biological activity relative to spinosad.*

Detailed pesticide use statistics are compiled by the state of California. The use statistics from California for 2013, the most recent year for which statistics are available, indicate a total use of spinosad of 34,771.95 lbs (CDPR 2015, pp. 691-695). No explicit forestry applications are noted in the California report. The use most closely related to forestry involved rights-of-way applications which consisted of a total of 2.68 pounds (i.e., about 0.0077% of total use). The only other use that might be relevant to Forest Service programs involved applications for landscape maintenance—i.e., 1453.07 lbs or about 4.2% of total use. Public health applications accounted for 6433.09 pounds or about 18.5% of total use. The relevance of this use to the Forest Service appears to be marginal since the Forest Service (Section 2.2) has not indicated that spinosad will be used in public health applications (e.g., mosquito control).

As with the USGS use statistics, CDPR notes that spinetoram use appears to be displacing spinosad:

> *Spinosad and spinetoram are primarily used in citrus to manage citrus thrips. Both are very selective, allowing natural enemies to survive. They may eventually erode the market share of older insecticides. Of the two, spinetoram is more effective against citrus thrips populations that have developed resistance to carbamate insecticides, and its persistence and effectiveness has resulted in the reduced use of spinosad. The area treated with spinosad decreased 55 percent in 2013, while spinetoram use increased 32 percent.*
>
> CDPR 2015, p. 114

Based on the use statistics from California, agricultural uses of spinosad would appear to be much greater than uses related to forestry or other non-agricultural applications. Because spinosad has not been used extensively in Forest Service programs or projects, however, it is

unclear at this time if Forest Service applications of spinosad would be negligible relative to agricultural applications.

3. HUMAN HEALTH

3.1. HAZARD IDENTIFICATION

3.1.1. Overview

Spinosad and other spinosyns act on the insect nervous system causing excitation of the neurons, primarily by the stimulation of nicotinic acetylcholine (nAChR) receptors and secondarily by the stimulation of gamma-aminobutyric acid (GABA) gated chloride channels. Spinosad, however, does not appear to be neurotoxic in mammals. The specific mechanism of toxicity for spinosad in mammals is not well-characterized but may involve the inhibition of P-glycoprotein, a cell constituent involved in the secretion of xenobiotics. One of the most common effects observed in animals treated with multiple doses of spinosad involves the development of cell vacuolation in many organs. It is not clear if this endpoint should be viewed as a frank sign of toxicity or an adaptive response. The EPA concluded that spinosad is "*Not likely to be Carcinogenic to Humans*". Spinosad does not appear to be specifically toxic to the fetus and has not been associated with birth defects. Adverse effects in offspring were noted (i.e., decreased litter size), but these effects appear to be secondary to maternal toxicity.

U.S. EPA's Office of Pesticide Programs (U.S. EPA/OPP) classifies potential acute hazards, based on several standard tests, ranging from the most hazardous (Category I) to the least hazardous (Category IV). U.S. EPA/OPP reviewed the acute toxicity data on spinosad and classified spinosad as Category IV based on acute oral, dermal, and inhalation toxicity. Spinosad is not a skin or eye irritant (Category IV). In addition, the EPA does not consider spinosad to be a skin sensitizer. Spinosad may cause mild irritation to the skin and eyes but has not been shown to cause skin sensitization. Spinosad is used by humans in the treatment of head lice. Consistent with the studies in mammals, the use of spinosad to treat head lice is associated with low incidences of mild irritation to the skin and eyes.

3.1.2. Mechanism of Action

The mechanism of action of spinosad in insects is relatively well understood (Section 4.1.2.4). Spinosad and other spinosyns act on the insect nervous system causing excitation of the neurons, primarily by the stimulation of nicotinic acetylcholine receptors (nAChR) and secondarily by the stimulation of gamma-aminobutyric acid (GABA) gated chloride channels (Barbosa et al. 2015; HSDB 2013; Thompson et al. 2015; U.S. EPA/OPP 2009a, 2012a; U.S. EPA/OPP/EFED 2005, 2011a). As discussed further in Section 3.1.6, however, spinosad and other spinosyns do not cause neurotoxic effects in mammals (U.S. EPA/OPP/HED 2011a), and incidents of human exposures to spinosad and other spinosyns are not associated with signs of neurotoxicity (U.S. EPA/OPP/HED 2011b).

As discussed in Section 2, spinosad consists of a macrolide ring system. Some macrolides, according to the review by Kanoh and Rubin (2010), are used clinically as immune modulators in the treatment of patients with various types of inflammatory diseases. As discussed further in Section 3.1.7, spinosad may impact immune function at high doses; however, this effect is not considered a sensitive or critical endpoint for exposure to spinosad. Although inflammatory changes are noted in some studies on both spinosad and spinetoram (U.S. EPA/OPP/2011a), these effects are not apparent in most toxicity studies on spinosad (Section 3.1.5).

One of the most common effects observed in animals treated with spinosad involves the development of cell vacuolation in many organs including the thyroid, parathyroid glands, liver, kidney and stomach. As the name implies, cytoplasmic vacuolation is the development of discrete membrane bound and morphologically distinct areas within a cell. Vacuolization is a general response associated with apoptosis (programmed cell death) as well as adaptation to limit cell damage (e.g., Henics and Wheatley 1999; Saikumar and Venkatachalam 2009). The FAO/WHO (2001) review of spinosad supports the assessment that cytoplasmic vacuolation following exposure to spinosad may be a reversible and adaptive response to stress.

There is some evidence that spinosad may enhance the neurotoxic effects of ivermectin by inhibiting P-glycoprotein transport (Section 3.1.16). P-glycoprotein is an ATP-dependent efflux pump involved in inhibiting the uptake and active secretion of xenobiotics from cells (Ambudkar et al. 2003). While this mechanism may be important in some drug interactions involving spinosad, the role of P-glycoprotein inhibition in the direct toxicity of spinosad is unclear.

3.1.3. Pharmacokinetics and Metabolism

3.1.3.1. Distribution and Metabolism

For pesticide registration, the U.S. EPA/OPP generally requires a relatively standard metabolism study in rats in which the compound is administered orally or by a combination of oral and intravenous routes (U.S. EPA/OPPTS 1998a). As summarized in both EPA documents and the review by FAO/WHO (2001), several metabolism studies are available on spinosad, all of which involve oral administration. The submissions to EPA are covered in greatest detail in U.S. EPA/OPP/HED 2009a. The primary study involves spinosyn A administered in single or multiple (14-day) doses of 10 mg/kg bw or single doses 100 mg/kg bw (MRIDs 43701508). Additional studies on spinosyn D involve single doses of 100 mg/kg bw/day (MRIDs 43701509 and 43701510). No remarkable differences in metabolism or distribution were noted between spinosyns A and D.

As would be expected of relatively lipophilic compounds, the spinosyns were primarily distributed to fat with substantial amounts also noted in kidneys, lymph nodes, and the thyroid. The open literature study by Rothwell et al. (2005) also notes substantial accumulation of spinosad in the fat of sheep. As discussed further in Section 3.1.5, the thyroid appears to be a target tissue following longer-term exposures to spinosad. As illustrated in Figure 4, spinosyns A and D undergo limited metabolism consisting of N-demethylation or O-demethylation as well as conjugation with glutathione. As discussed further in Section 4.1.2.2, N-demethylation and O-demethylation also appear to be common metabolic processes in birds (Magnussen et al. 1996).

While information on the toxicity of spinosad metabolites to mammals is not available, the *in vivo* metabolites as well as environmental metabolites (Section 3.1.15.1) are similar to the parent compound, and the EPA assumes that the metabolites are similar in toxicity to the parent compounds—i.e., spinosyns A and D. As discussed further in Section 4.1.3.3 (hazard identification for aquatic invertebrates), the limited toxicity data on the metabolites of spinosad suggest that the metabolites are comparable in toxicity to the parent compounds (U.S. EPA/OPP/EFED 2009b).

3.1.3.2. Dermal Absorption

Most of the occupational exposure scenarios and many of the exposure scenarios for the general public involve the dermal route of exposure. For these exposure scenarios, dermal absorption is estimated and compared to an estimated acceptable level of oral exposure based on subchronic or chronic toxicity studies in animals. It is, therefore, necessary to assess the consequences of dermal exposure relative to oral exposure and the extent to which spinosad is likely to be absorbed from the skin surface.

Two types of dermal exposure scenarios are considered: immersion and accidental spills. In the scenarios involving immersion, the concentration of the chemical in contact with the surface of the skin is assumed to remain constant or at least nearly so. As detailed in SERA (2014a), the calculation of absorbed dose for dermal exposure scenarios involving immersion requires an estimate of the dermal permeability coefficient (K_p) expressed in cm/hour, and the rate of absorption is assumed to be essentially constant (i.e., zero-order kinetics as discussed in Section 3.1.3.2.2). In exposure scenarios involving direct sprays or accidental spills where the compound is deposited directly on the skin, the concentration or amount of the chemical on the surface of the skin is assumed to be the limiting factor in dermal absorption. For these scenarios first-order dermal absorption rate coefficients (k_a), expressed as a proportion of the deposited dose absorbed per unit time—e.g., $hour^{-1}$—are used in the exposure assessment.

3.1.3.2.1. First-Order Dermal Absorption

The EPA human health risk assessments on spinosad (i.e., U.S. EPA/OPP/HED 1997a,b, 2007a, 2009a, 2009b, 2010a,b, 2011a) do not address the dermal absorption of spinosyns. The only semi-quantitative note on dermal absorption is found in U.S. EPA/OPP/HED 1997b, p. 13: "*If there is a need for a chronic risk assessment, a factor of no greater than 10% should be used for dermal absorption*". It should be noted that the "factor" referenced by EPA is not a first-order dermal absorption rate coefficient but rather the percent absorbed over a work day, typically taken as 8 hours. Thus, an absorption factor of 10% would be equivalent to a first-order dermal absorption rate coefficient of about 0.013 $hour^{-1}$ [$\ln(1\text{-}0.1) \div 8$ hours ≈ 0.01317 $hour^{-1}$].

The more recent EPA risk assessments simply note that spinosad is not likely to pose a risk in dermal exposures:

> *Short-, intermediate-, and long-term dermal risk assessments are not required for the following reasons: 1) lack of concern for pre and/or post natal toxicity; 2) the combination of molecular structure and size as well as the lack of dermal or systemic toxicity at 1000 mg/kg/day in a 21-day spinosad and spinetoram dermal toxicity studies in rats which indicates poor dermal absorption; and 3) the lack of long-term exposure based on the current use pattern.*
>
> U.S. EPA/OPP/HED 2011a

Other recent EPA risk assessments, cited above, contain similar language. The 21-day dermal toxicity study noted in the above EPA quotation is discussed further with other dermal toxicity studies in Section 3.1.12 (Systemic Toxic Effects from Dermal Exposure). As discussed further in Section 3.2.2.1, the U.S. EPA does not consider dermal exposure in the worker exposure assessment.

While the EPA risk assessments do not discuss dermal absorption studies on spinosad, the FAO/WHO (2001) review of spinosad briefly summarizes a dermal absorption study on spinosyn A in rats that was submitted to WHO by Dow AgroSciences, United Kingdom. In this study, cited in FAO/WHO (2001) as Domoradzki and Shabrang 1996, 1% of dermally applied spinosyn A was absorbed by rats over a 24 hour exposure period. Assuming first-order absorption, these results correspond to a first-order dermal absorption rate coefficient of about 0.00042 $hour^{-1}$ [ln(1-0.01÷24 hours ≈ 0.0004188 $hour^{-1}$].

Forest Service risk assessments typically consider the use of quantitative structure activity relationships (QSAR), as detailed in SERA (2014a, Section 3.1.3.2.2). The QSAR method is based exclusively on dermal absorption data from studies in humans involving numerous chemicals. As detailed in Worksheet B03b of Attachments 1 and 2, the QSAR methods yield estimated dermal absorption rate coefficients for spinosyn A of about 0.00002 (0.0000007–0.0005) $hour^{-1}$ using a K_{ow} value of 10,000 and a molecular weight of 731.98 (Table 1 with values taken from U.S. EPA/OPP/HED 2011a). While the K_{ow} for spinosyn A is within the range of values on which the algorithm is based—i.e., K_{ow} values ranging from 0.0015 to 3,000,000—the molecular weight of spinosyn A exceeds the range of molecular weights on which the algorithm is based—i.e., 60 to 400 g/mole.

The current Forest Service risk assessment uses the estimated dermal absorption rate coefficients of 0.00002 (0.0000007–0.0005) $hour^{-1}$ based on the QSAR method from SERA (2014a, Section 3.1.3.2.2). While the high molecular weight of spinosyn A diminishes confidence in the estimates from the QSAR algorithm, it should be noted that the upper bound of the estimate does encompass the first-order dermal absorption rate coefficient in rats of about 0.00042 $hour^{-1}$ from the study by Domoradzki and Shabrang (1996). More significantly, as noted above, the algorithm from SERA (2014a) is based on human data. As reviewed by Ravenzwaay and Leibold (2004), rat skin is more permeable than human skin by about a factor of about 10. Thus, the rate coefficient of 0.00042 $hour^{-1}$ in rats would suggest a comparable rate coefficient in humans of about 0.00004 $hour^{-1}$, which is close to the central estimate of 0.00002 $hour^{-1}$ from the QSAR algorithm.

While the current Forest Service risk assessment does not adopt the same approach used by EPA—i.e., dermal exposure is negligible—this difference from EPA does not materially impact the current risk assessment. The dermal absorption rate coefficients of 0.00002 (0.0000007–0.0005) $hour^{-1}$ are extremely low. As discussed further in Section 3.4 (risk characterization), none of the dermal exposures for workers or members of the general public approaches a level of concern, which is consistent with the EPA risk characterizations.

3.1.3.2.2. Zero-Order Dermal Absorption

Exposure scenarios involving the assumption of zero-order dermal absorption require an estimate of dermal permeability (K_p) in units of cm/hour. No experimental data are available on the dermal permeability rate of spinosad as a mixture or on spinosyns A or D. In the absence of experimental data, Forest Service risk assessments generally use a QSAR algorithm developed by the EPA (U.S. EPA/ORD 1992, 2007). This approach is discussed in further detail in SERA (2014a, Section 3.1.3.2.1). As with the algorithm for estimating the first-order dermal absorption rate constant, the EPA algorithm is based on molecular weight and K_{ow} values (U.S. EPA/ORD

1992, 2007). The molecular weight and K_{ow} values used for estimating the K_p are identical to those used in the estimate of the first-order dermal absorption rate constants (i.e., a K_{ow} value of 10,000 and a molecular weight of 731.98). The EPA algorithm is derived from an analysis of 95 organic compounds with K_{ow} values ranging from about 0.0056 to 309,000 and molecular weights ranging from approximately 30 to 770 (U.S. EPA/ORD 1992, 2007). These ranges of K_{ow} values and molecular weights encompass the estimates of the corresponding values for spinosyn A.

Details of the implementation of the algorithms are given in Worksheet B03a in the EXCEL workbooks for spinosad (Attachments 1 and 2). Using the EPA algorithm results in an estimated dermal permeability (K_p) of about 0.00004 (0.00001 to 0.0001) cm/hour.

3.1.3.3. Excretion

Although excretion rates are not used directly in either the dose-response assessment or risk characterization, excretion half-lives can be used to infer the effect of longer-term exposures on body burden, based on the *plateau principle* (e.g., Goldstein et al. 1974, p. 320 ff). Under the assumption of first-order elimination, the first-order elimination rate coefficient (***k***) is inversely related to the half-life ($\boldsymbol{T_{50}}$) [$\boldsymbol{k} = \ln(2) \div \boldsymbol{T_{50}}$]. If a chemical with a first-order elimination rate constant of ***k*** is administered at fixed time interval (***t****) between doses, the body burden after the $\boldsymbol{N^{th}}$ dose ($\boldsymbol{X_{N\ Dose}}$) relative to the body burden immediately following the first dose ($\boldsymbol{X_{1\ Dose}}$) is:

$$\frac{X_{N\ Dose}}{X_{1Dose}} = \frac{(1-\left(e^{-kt^*}\right)^N)}{1-e^{-kt^*}} \qquad (1)$$

As the number of doses (***N***) increases, the numerator in the above equation approaches a value of 1. Over an infinite period of time, the plateau or steady-state body burden ($\boldsymbol{X_{Inf}}$) can be calculated as:

$$\frac{X_{Inf}}{X_1} = \frac{1}{1-e^{-kt^*}} \qquad (2)$$

Whole-body half-lives are most appropriate for estimating steady-state body burdens.

In standard metabolism studies in rats, biphasic excretion kinetics are noted with an initial half-life of 0.25 days and a terminal half-life of 1.25 days (MRID 43701509, U.S. EPA/OPP/HED 2009a). Somewhat longer half-lives are reported in the open literature for dogs—i.e., a terminal plasma half-life of about 11.2 days in the study by Dunn et al. (2011) and mean elimination half-times of 7 to 10 days in the study by Holstrom et al. (2012).

The terminal half-life of 1.25 day for rats corresponds to an elimination rate coefficient of about 0.55 day^{-1} [$\ln(2) \div 1.25$ days ≈ 0.5545 day^{-1}]. Substituting this rate coefficient into the above equation, the estimated plateau for rats is about 2.4 [$1 \div (1-e^{-0.55}) \approx 2.363$]. Taking the elimination half-time for dogs at about 10 days, the elimination rate coefficient for dogs is about 0.07 day^{-1} [$\ln(2) \div 10$ days ≈ 0.0693 day^{-1}]. Substituting this rate coefficient for dogs into the above equation, the estimated plateau for dogs is about 15 [$1 \div (1-e^{-0.07}) \approx 14.7$]. Thus, dogs may be expected to accumulate spinosad to a greater extent than rats by about a factor of 6 [$15 \div 2.4 =$

6.25]. As discussed further in Section 3.1.5 (subchronic and chronic toxicity), dogs appear to be more sensitive than rats to spinosad. It seems only modestly speculative to suggest that the greater sensitivity in dogs may be due to the slower elimination of spinosad by dogs relative to rodents.

As discussed further in Section 3.1.5, the LOAELs for dogs following subchronic and chronic exposures do not differ remarkably – i.e., the subchronic (90 day) LOAEL is about 10.1 mg/kg bw and the chronic (1 year) LOAEL is 8.34 mg/kg bw/day. As also discussed in Goldstein et al. (1974, p. 321), the fractional value of the eventual steady state (*f*) can be calculated as:

$$f = 1 - e^{-kt*n} \quad (3)$$

Based on the above equation and the elimination rate coefficient for dogs of 0.07 day^{-1}, dogs would reach about 0.998 of the eventual plateau by 90 days [1-e^-0.07*90 ≈ 0.998164]. Consequently, there would be no substantial difference in body burden for a dog following exposures of 90 days (subchronic exposure) and 1 year (chronic exposure). Thus, the similarities between the subchronic and chronic LOAELs for dogs are consistent with the apparent excretion kinetics of spinosad in dogs.

3.1.4. Acute Oral Toxicity

3.1.4.1. Standard Registrant Studies

Standard acute oral toxicity studies are typically used to determine LD_{50} values—i.e., the treatment dose estimated to be lethal to 50% of the animals. LD_{50} values are not used directly to derive toxicity values as part of the dose-response assessment in Forest Service risk assessments. LD_{50} values as well as other measures of acute toxicity discussed in following sections are used by the U.S. EPA/OPP to categorize potential risks. U.S. EPA/OPP uses a ranking system for responses ranging from Category I (most severe response) to Category IV (least severe response). Details of the EPA system of categorization are detailed in SERA (2014a, Table 4) as well as in U.S. EPA/OPP (2015b, Table 1), the label review manual.

Acute oral LD_{50} values for spinosad are summarized in Appendix 1, Table A1-1. All of the acute oral toxicity studies appear to involve technical grade spinosad. The EPA classifies spinosad as Category III (LD_{50} >500 mg/kg bw, <5000 mg/kg bw) for acute oral toxicity based on the acute oral LD_{50} values of >2000 mg a.i./kg bw in rats for technical grade spinosad (U.S. EPA/OPP/HED 2009a, Attachment 2, MRID 00132519).

A definitive LD_{50} value of 3738 mg/kg bw is reported for male rats. This LD_{50} value is reported in three different sources, each of which provides somewhat different details in terms of experimental design or study attribution. The DER from EPA attributes this study to Gilbert et al. (1994, MRID 43414515) while FAO/WHO (2001) attributes this study to Stebbins and Brooks (1999a). This minor discrepancy probably reflects differences in submissions of the study by the registrant to EPA and WHO. The summary of this study in U.S. EPA/OPP/HED (1997b) notes that the reported LD_{50} of 3738 mg a.i./kg bw appears to be a combination of the data from two different submissions—i.e., Gilbert et al. 1994 (MRID 43414515) and Wright et al. 1992 (MRIDs 43770701 and 43414515). This supposition appears to be correct in that the

study by Gilbert et al. (1994) involved a single dose of 5000 mg a.i./kg bw and the study by Wright et al. (1992) involved a single dose of 5000 mg/kg bw. Thus, the reported definitive LD_{50} involves zero degrees of freedom.

The only other definitive LD_{50} values reported for spinosad are the LD_{50} values of 6100 mg/kg bw in male mice and 7100 mg/kg bw in female mice. These definitive LD_{50} values are reported in the FAO/WHO (2001) review and are attributed to a study by Gilbert and Yano (1996). The EPA risk assessments on spinosad report only an indefinite LD_{50} of >5000 mg/kg bw (Gilbert et al. 1994 MRID 43414515). The study by Gilbert et al. (1994, MRID 43414515) is a limit test that involved only a single dose, which means it could not have been used to estimate the definitive LD_{50} values reported in FAO/WHO (2001). Given the lack of detail in the FAO/WHO (2001) summary and the limitations in the definitive LD_{50} for rats (discussed above), the differences in the reported definitive LD_{50} values in rats and mice cannot be overly interpreted in terms of differences in species sensitivity.

All of the MSDS for the representative formulations specify acute oral LD_{50} values of >5000 mg/kg bw for rats (Table 4). This toxicity value is consistent with the LD_{50} for female rats from MRID 43414515 (Appendix 1, Table A1-1) but not with some of the lower LD_{50} values. The specification of the LD_{50} of >5000 mg/kg bw/day in mice is consistent with Gilbert et al. (1994, MRID 43414515) as well as Gilbert and Yano (1996 as summarized in FAO/WHO 2001).

3.1.4.2. Other Data

One human poisoning incident is reported in the open literature (Su et al. 2011). The incident occurred in Taipei, Taiwan and was associated with a suicide attempt in which an 80-year old woman consumed both 80 mL of Conserve (11.6% spinosad or about 9 g a.i.) as well as 2 to 3 grams of flonicamid. The woman evidenced signs of neurotoxicity within 3 hours of dosing and recovered after prompt medical treatment. Like spinosad, flonicamid is neurotoxic to insects but does not cause signs of neurotoxicity in humans. Flonicamid is somewhat more toxic to rats than spinosad with acute oral LD_{50} values of 884 mg/kg bw in male rats and 1768 mg/kg bw in female rats (U.S. EPA/OPP/HED 2014a). The body weight of the woman is not specified in the report from Su et al. (2011). Taking 50 kg as an approximate weight of a female from Taiwan (Tao 2014), the woman may have consumed a dose of 180 mg/kg bw of spinosad or about 5% of the rat oral LD_{50} [180 mg/kg bw ÷ 3738 mg a.i./kg bw ≈ 4.8154%] and a dose of 50 mg/kg bw [2500 mg ÷ 50 kg] of flonicamid or about 3% of the LD_{50} for female rats [50 mg/kg bw ÷ 1768 mg/kg bw ≈ 2.82895]. Consistent with the discussion by Su et al. (2011), these dose estimates would not clearly indicate the involvement of either spinosad or flonicamid in the effects on the patient and suggest that other ingredients in the formulation may have contributed to the adverse effects.

In addition to the poisoning incident in Taiwan, the U.S. EPA/OPP/HED (2011b) reviewed human incidents involving spinosad in the OPP Incident Data System. The EPA review provides few details but does note that six incidents involving spinosad have been reported and that …*most are of lower severity* (U.S. EPA/OPP/HED 2011b, p. 3). Additional details of the effects noted and levels of exposure are not provided.

Spinosad is used for the treatment of fleas in both cats and dogs. Adverse effects following oral doses in the range of 50 to about 100 mg/kg bw have been associated with vomiting in some studies (Elanco 2012; Paarlberg et al. 2013; Elanco Animal Health 2007). Other studies indicate

no adverse effects over this dose range (Snyder et al. 2013; Franc and Bouhsira 2009; Wolken et al. 2012). At a dose of up to 300 mg/kg bw (five times the maximum labelled dose), collies evidenced no adverse effects other than vomiting, and none of the collies required supportive treatment (Sherman et al. 2010).

3.1.5. Subchronic or Chronic Systemic Toxic Effects

As discussed in SERA (2014a, Section 3.1.5), *subchronic* and c*hronic* are somewhat general terms that refer to studies involving repeated dosing. Some repeated dose studies are designed to detect specific toxic endpoints, like reproductive and neurological effects. These more specialized studies involving multiple dosing are discussed in subsequent subsections of this hazard identification except for some comments in this subsection on general signs of toxicity.

The subchronic and chronic toxicity studies on spinosad are summarized in Appendix 1, Table A1-2. Most of the studies relevant to the current risk assessment were submitted to the U.S. EPA/OPP in support of the registration of spinosad, and the summaries of these studies are taken from EPA human health risk assessments (U.S. EPA/OPP/HED 1997a, 2009a, 2014a). Some repeated dose studies published in the open literature are from the Dow Chemical Company and appear to be identical to studies submitted to EPA, as specified in Appendix 1, Table A1-2 (i.e., Stebbins et al. 2002; Yano et al. 2002). The subchronic study in rats by El-Hoda et al. (2012) focuses on cellular aberrations in bone marrow, as discussed further in Section 3.1.10 (Mutagenicity and Carcinogenicity). The FAO/WHO (2001) review of spinosad provides detailed summaries of several subchronic and chronic studies. As discussed below, some of these studies do not appear to have been submitted to EPA, and some of these studies provide some insight on the similarities of various blends of spinosyns A and D in spinosad.

Relatively standard studies regarding the subchronic toxicity of spinosad in dogs are available (MRID 43444102), mice (MRID 43566602; Stebbins et al. 2002), and rats (MRID 43566601; Wilmer et al. 1993, MRID 43557504). Standard chronic toxicity studies are also available on these species—i.e., dogs (Harada 1995, MRID 43701504), mice (Bond et al. 1995a, MRID 43701505; MRID 44123601), and rats (Bond et al. 1995b, MRIDs 43701507 and 43710503; Spencer and Yano 1995, MRID 43701507 and 43701503).

As noted in Section 3.1.2 (Mechanism of Action), one of the most common signs of subchronic or chronic exposure to spinosad involves cytoplasmic vacuolation in the cells of many organs, including the thyroid, parathyroid glands, liver, kidney, and stomach. This effect is noted specifically in subchronic and/or chronic studies in mice (MRID 43566602; Bond et al. 1995a, MRID 43701505), rats (MRID 43566601, Bond et al. 1995b, MRIDs 43701507 and 43710503), and dogs (Harada 1995, MRID 43701504).

In terms of species differences following subchronic or chronic exposures to spinosad, dogs appear to be somewhat more sensitive than rodents. The data on subchronic and chronic NOAELs and LOAELs for mice, rats, and dogs are summarized in Table 6 and illustrated in Figure 5. The additional details of these studies are summarized in Appendix 1 (Table A1-2). For studies that provide separate NOAELs and LOAELs for males and females, the NOAELs and LOAELs given in Table 6 are presented as the arithmetic average of the values for males and females, with all values rounded to the nearest tenth.

Based on the data summarized in Table 6, dogs appear to be more sensitive than either mice or rats in terms of both NOAELs and LOAELs from subchronic and chronic studies. Based on subchronic LOAELs in beagle dogs (LOAEL of 10.1 mg/kg bw/day, MRID 43444102) and LOAELs in rats (73 mg/kg bw/day, MRID 43566601), dogs are more sensitive than rats by a factor of about 7 [73 mg/kg bw/day ÷ 10.1 mg/kg bw/day ≈ 7.222…]. As discussed in Section 3.1.3.3, this difference in apparent sensitivity is consistent with the slower excretion kinetics in dogs relative to rats suggesting that dogs may accumulate more spinosad than rats by about a factor of 6. This correspondence, however, may be coincidental. Based on chronic LOAELs of about 8.34 mg/kg bw/day in dogs (Harada 1995, MRID 43701504) and 27.3 mg/kg bw/day in rats (Bond et al. 1995b, MRIDs 43701507 and 43710503), the difference in sensitivity between dogs and rats is only a factor of about 3 [27.3 mg/kg bw/day ÷ 8.34 mg/kg bw/day ≈ 3.27].

NOAELs and LOAELs are not generally appropriate for quantitative analysis because they are based on experimental doses rather than modelled estimates of equitoxic responses (e.g., LD_{50} values). In addition, the designation of NOAELs and LOAELs can be judgmental. For example, as noted in Appendix 1, Table A1-2, the most recent EPA risk assessment reevaluates the chronic study in rats (Bond et al. 1995b, MRIDs 43701507 and 43710503) and reclassifies the NOAEL designation for 3 mg/kg bw/day to 9.5 mg/kg bw/day. These types of reevaluations are not uncommon, and, following standard practice in Forest Service risk assessments, the most recent EPA designations are used in the current risk assessment.

With the above reservations, which are substantial, and as illustrated in Figure 5, the chronic LOAELs in mice, rats, and dogs appear to reflect a systematic difference in toxicity. As an exploratory effort, these chronic LOAELs were fit to a standard allometric equation with the following parameters:

$$LOAEL = 17 \times BW^{-0.315} \quad (4)$$

Even though only three data points are available (i.e., a single degree of freedom for the two-parameter model), the fit to the model is statistically significant ($p \approx 0.0091$) with a high correlation coefficient ($r^2=0.9998$). As discussed further in Section 3.3.5 (Dose-Severity Relationships), the above equation leads to an estimated LOAEL of about 4.5 mg/kg bw/day [$17 \times 70^{-0.315} \approx 4.4591$] for a 70 kg mammal (i.e., a standard body weight for humans). While statistically significant for chronic studies, the allometric relationship is not reflected in the subchronic studies where the LOAEL for mice is below the LOAEL for rats by a factor of about 3 [73 mg/kg bw/day ÷ 22.5 mg/kg bw/day ≈ 3.2444…]. Nonetheless, and consistent with the more qualitative EPA analyses (U.S. EPA/OPP/HED 2009a, 2011b), dogs are identified as the most sensitive species of mammals based on the available subchronic and chronic studies and are used in the dose-response assessment for potential human health effects (Section 3.3).

Another noteworthy relationship in the subchronic and chronic studies is the similarity of the subchronic NOAELs and LOAELs in dogs (5.1/10.1 mg/kg bw/day) to the chronic NOAELs and LOAELs in dogs (2.7/8.34 mg/kg bw/day). As discussed in Section 3.1.3.3, the proximity of the subchronic and chronic toxicity values is consistent with the elimination rate coefficient of spinosad in dogs.

As discussed in Section 2.2, the proportion of spinosyns A and D in spinosad is variable, ranging from about 1:1::A:D to 5.7:1::A:D. The registrant studies summarized in EPA risk assessments do not generally provide information on the ratios of spinosyns A and D in spinosad. The review by FAO/WHO (2001) does summarize the results of two subchronic studies in rats, one study using a 1:1 ratio of spinosyn A to spinosyn D and the other using a 5:1 ratio of spinosyn A to spinosyn D. The study using the 1:1 mixture reports a LOAEL of 39 mg/kg bw/day in males and 47 mg/kg bw/day in females. The study using the 5:1 mixture reported a LOAEL of 34 mg/kg bw/day in males and 39 mg/kg bw/day in females. While this is an extremely limited basis for comparison, these studies suggest no substantial differences in the toxicity of spinosad over ranges of spinosyn A to spinosyn D commonly found in commercial formulations.

In addition to the studies on spinosad, Appendix 1, Table A1-2 also summarizes a subchronic toxicity study on spinetoram in dogs (MRID 47011901). Like spinosad, spinetoram is a mixture of two spinosyns (J and L) which are also fermentation products of *Saccharopolyspora spinosa*. Unlike spinosad, spinosyns J and L are chemically modified in the production of spinetoram (Dow 2014b). Nonetheless, the components in spinetoram are structural analogues to the spinosyns in spinosad. Based on structural similarities and similar toxicological action, spinosad and spinetoram are considered toxicologically equivalent by EPA, at least in terms of human health effects (U.S. EPA/OPP/HED 2009a, 2011a). As discussed further in Section 3.3 (Dose-Response Assessment), the EPA derives the chronic RfD for spinosad based on the chronic study of spinetoram in dogs (MRID 47011901). As summarized in Appendix 1 (Table A1-2), the chronic NOAELs of spinetoram in male dogs (2.96 mg/kg bw/day and female dogs (2.49 mg/kg bw/day) are virtually identical to the chronic NOAELs of spinosad in male dogs (2.66 mg/kg bw/day and female dogs (2.71 mg/kg bw/day). While the current Forest Service risk assessment does not encompass the toxicity studies of spinetoram explicitly, the assessment by U.S. EPA/OPP/HED (2009a, 2011a) on the toxicological equivalence of spinosad and spinetoram seems reasonable. As discussed further in Section 3.3, the current risk assessment defers to EPA on the selection of the spinetoram study as the basis for the chronic RfD for spinosad. As noted above, the NOAELs for spinosad and spinetoram are virtually identical, and the use of the spinetoram study rather than the spinosad study does not materially impact the risk assessment.

3.1.6. Effects on Nervous System

As discussed in Section 4.1.2.4, neurotoxicity is considered the primary endpoint of concern for terrestrial invertebrates. This is not the case for the human health risk assessment. As summarized in Appendix 1 (Tables A1-1 and A1-2), spinosad has been subject to an acute neurotoxicity study (Albee et al. 1994, MRIDs 43557501) and a subchronic neurotoxicity study in rats (Wilmer et al. 1993, MRID 43557504) in rats. In addition, neurotoxicity studies (functional observational batteries) were conducted at months 3, 6, 9, and 12 of the chronic toxicity study in rats (Spencer and Yano 1995, MRID 43701507 and 43701503). The acute and subchronic neurotoxicity studies noted no signs of toxicity (neurotoxic or otherwise) at a dose of 2000 mg/kg bw in the acute study and doses of up to 42.7 mg/kg bw/day in males and 52.1 mg/kg bw/day in females in the subchronic study. In the chronic study, no signs of neurotoxicity were noted at doses of up to 46.0 mg/kg/day in male and 57.0 mg/kg/day in female rats. Based on these results and consistent with the assessment from U.S. EPA/OPP/HED (2011a, p. 6), neurotoxicity in mammals is not considered an endpoint of concern in the human health risk assessment.

3.1.7. Effects on Immune System

There are various methods for assessing the effects of chemical exposure on immune responses, including assays of antibody-antigen reactions, changes in the activity of specific types of lymphoid cells, and assessments of changes in the susceptibility of exposed animals to resist infection from pathogens or proliferation of tumor cells. Typical subchronic or chronic animal bioassays involve morphological assessments of the major lymphoid tissues, including bone marrow, major lymph nodes, spleen and thymus (organ weights are sometimes measured as well), and blood leukocyte counts. These assessments can detect signs of inflammation or injury indicative of a direct toxic effect of the chemical on the lymphoid tissue. Changes in morphology of lymphoid tissue and blood, indicative of a possible immune system stimulation or suppression, can also be detected.

As summarized in Appendix 1 (Tables A1-2 and A1-3), histopathology and/or changes in organ weight were observed in the spleen (rats in Breslin et al. 1994, MRIDs 43701506) and bone marrow (mice in MRID 43566602; rats in El-Hoda et al. 2012) following subchronic exposures to spinosad and in the thymus following subchronic exposure to spinetoram (dogs in MRID 47011901). These changes in tissues associated with the immune system were …*considered secondary to a systemic inflammatory reaction* (U.S. EPA/OPP/HED 2011a, p. 6). The EPA also notes: *A non-statistically significant decrease in the anti-Susquehanna River Basin Commission (SRBC) response was also observed in the high dose group*. The term *Susquehanna River Basin Commission* appears to be a simple error in the definition of SRBC (Sheep Red Blood Cells).

As also summarized in the most recent EPA human health risk assessment, the EPA requested and received an immunotoxicity study on spinosad. Details of this study are not available but the EPA summary indicates that a spinosad dose of 141 mg/kg bw/day (species not given) resulted in an increase in neutrophils and monocytes and a decrease in lymphocytes (U.S. EPA/OPP/HED 2011a, p. 5). Because these effects were noted only at a high dose, the EPA suggested that concern for immunotoxicity is low. Immunotoxicity is not addressed in the various reviews of spinosad from the European literature (EFSA 2011, 2012, 2013, 2014; European Commission 2006; FAO/WHO 2001; WHO 2011) or the recent APHIS human health risk assessment (APHIS 2014).

In the absence of additional details on the specific immunotoxicity studies on spinosad, the current Forest Service risk assessment defers to the judgement of U.S. EPA/OPP/HED (2011a), and immunotoxicity is not identified as an endpoint of primary concern for spinosad.

3.1.8. Effects on Endocrine System

Assessments of the direct effects of chemicals on endocrine function are most often based on mechanistic studies on estrogen, androgen, or thyroid hormone systems (i.e., assessments on hormone synthesis, hormone receptor binding, or post-receptor processing). In addition, inferences concerning the potential for endocrine disruption can sometimes be made from responses seen in standard toxicity tests—i.e., changes in the structure of major endocrine glands (i.e., the adrenal, hypothalamus, pancreas, parathyroid, pituitary, thyroid, ovary, and testis) or changes in growth rates. Effects on organs associated with endocrine function may be secondary to other toxic effects. Thus, in the absence of information on specific endocrine mechanisms,

pathological changes in endocrine tissues do not necessarily indicate a direct effect on endocrine function.

As summarized in Appendix 1 (Table A1-2), changes in endocrine glands were observed in several subchronic and chronic studies. These changes include vacuolation of the adrenals and pancreas in mice (MRID 43566602), the adrenals in rats (MRID 43566601), and the parathyroid in dogs (Harada 1995, MRID 43701504). Effects on the thyroid include both changes in histopathology in rats (MRID 43566601; Bond et al. 1995b, MRIDs 43701507 and 43710503) and dogs (MRID 47011901, spinetoram only) as well as increased thyroid weights in rats (MRID 43566601) and dogs (Harada 1995, MRID 43701504). Increases in thyroid weights were also observed in rats in a reproduction study (Breslin et al. 1994, MRIDs 43701506; Hanley et al. 2002), which is discussed further in Section 3.1.9.2. No effects were observered in ovaries or testes.

As noted in U.S. EPA/OPP/HED (2011a, p. 6), both spinosad and spinetoram are subject to endocrine screening as part of the EPA's Endocrine Disruptor Screening Program. Neither spinosad nor spinetoram have been tested to date, based on information available at the EPA web site for the Endocrine Disruptor Screening Program (https://www.epa.gov/ingredients-used-pesticide-products/endocrine-disruptor-screening-program-tier-1-assessments). This status is also noted in the recent USDA/APHIS human health risk assessment on spinosad (USDA/APHIS 2014, p. 6). Potential effects on the endocrine system are not addressed in the various spinosad reviews from the European literature (EFSA 2011, 2012, 2013, 2014; European Commission 2006; FAO/WHO 2001; WHO 2011).

In terms of functional effects that have important public health implications, effects on endocrine function could be expressed as diminished reproductive capacity in adults or abnormal fetal development. As discussed in the following section (Section 3.1.9), spinosad does not appear to be associated with specific adverse effects on either fetal development or reproductive performance. Based on these data, the EPA indicated that … *concern for endocrine-related effects is low* (U.S. EPA/OPP/HED 2009a, p. 5). In the absence of mechanistic studies or other clear evidence of disruptions in endocrine function, the current Forest Service risk assessment concurs with the EPA assessments, and effects on endocrine function are not identified as endpoints of primary concern for spinosad.

One inconsistency in the literature concerns the reproduction study in rats, which is discussed further in Section 3.1.9.1. This study was submitted to the EPA (Breslin et al. 1994, MRIDs 43701506) and is published in the open literature (Hanley et al. 2002). The EPA summary of this study indicates that the high dose (100 mg/kg bw/day) resulted in …*cytoplasmic vacuolation of the follicular epithelial cells of the thyroid with increased levels of thyroid-stimulating hormone (TSH) and decreased levels of* T_4 (U.S. EPA/OPP/2009a, p. 5). The open literature publication provides a summary of the data (Hanley et al. 2002, Table 4) indicating no change in T_4 levels. Assays of TSH are not discussed in the publication.

3.1.9. Reproductive and Developmental Effects

3.1.9.1. Developmental Studies

Developmental studies are used to assess the potential of a compound to cause malformations and signs of toxicity during fetal development. These studies typically entail gavage administration of the chemical compound to pregnant rats or rabbits on specific days of gestation. Teratology assays as well as studies on reproductive function (Section 3.1.9.2) are generally required by the EPA for the registration of pesticides, and specific protocols are established by EPA for developmental and reproduction studies (U.S. EPA/OPPTS 2000).

As summarized in Appendix 1, Table A1-3, standard developmental toxicity studies were conducted in rats (Liberacki et al. 1993, MRIDs 43557505 and 43770702) and rabbits (Vedula et al. 1994, MRIDs 43414521 and 43770703). These registrant-submitted studies are also published in the open literature (Breslin et al. 2000; Marty et al. 1998). Developmental effects are not noted in either study. No signs of systemic maternal toxicity were observed in rats at a dose of up to 200 mg/kg bw/day. Marginal and statistically insignificant signs of maternal toxicity were observed in rabbits at the highest dose tested, 50 mg/kg bw/day—i.e., an increase in the incidence of decreased defecation and a transient (Days 7-10) decrease in body weight gain (28% less than controls). The EPA judged these effects to be not toxicologically significant and classifies 50 mg/kg bw/day as a NOAEL for maternal toxicity in rabbits (U.S. EPA/OPP/HED 2009a, p. 45).

The EPA evaluation is consistent with the FDA classification of spinosad as a Category B drug (Shmidt and Levitt 2012)—i.e., *Animal reproduction studies have failed to demonstrate a risk to the fetus* … (https://chemm.nlm.nih.gov/pregnancycategories.htm).

Given the lack of fetal toxicity and developmental effects in the studies on rats and rabbits and the determinations by EPA and FDA, developmental effects are not considered an endpoint of substantial concern for spinosad.

3.1.9.2. Reproduction Studies

Reproduction studies involve exposing one or more generations of the test animal to a chemical compound. Generally, the experimental method involves exposing one or more parental (P_i) generations to the test substance prior to mating, during mating, after mating, and through weaning of the offspring (F_1). In a 2-generation reproduction study, this procedure is repeated with male and female offspring from the F_1 generation to produce another set of offspring (F_2). In the case of spinosad, the reproduction study (discussed below) involved the generation of two groups of offspring from the P_1 generation—i.e., F_{1a} and F_{1b} offspring—with the F_{1a} offspring acting as the P_2 generation to produce a F_2 offspring. During these types of studies, standard observations for gross signs of toxicity are made. Additional observations often include the length of the estrous cycle, assays on sperm and other reproductive tissue, and number, viability, and growth of offspring. Typically, the EPA requires one acceptable multi-generation reproduction study for pesticide registration (U.S. EPA/OPPTS 2000).

As summarized in Appendix 1 (Table A1-3), one standard two-generation reproduction study was submitted to EPA in support of the registration of spinosad (Breslin et al. 1994, MRIDs 43701506). A summary of this study is also published in the open literature (Hanley et al. 2002).

In this study, the parental generations were dosed at 0, 3, 10, or 100 mg/kg bw for 10 weeks (P_1 generation) or 12 weeks (P_2 generation). No adverse effects were seen at the two lower doses. At 100 mg/kg bw/day, reduced body weight, increases in relative and absolute weights of heart, kidney, liver, spleen, and thyroid as well as multiple organ pathology (specified in Appendix 1) were observed. In addition, delivery complications resulting in the death of five females were observed at 100 mg/kg bw. Adverse effects in offspring, including smaller litter size/fetal mortality were observed and appeared to be related to maternal toxicity. Based on this study, reproductive effects are considered endpoints of concern but are not considered the most sensitive endpoint. As discussed further in Section 3.3, the NOAELs used for the dose response assessments are substantially below the NOAEL of 10 mg/kg bw/day for reproductive effects.

3.1.10. Carcinogenicity and Mutagenicity

As summarized in Appendix 1 (Table A1-2), standard chronic carcinogenicity studies were conducted in mice (Bond et al. 1995a, MRID 43701505) and rats (Bond et al. 1995b, MRIDs 43701507 and 43710503). These studies are also published in the open literature as Stebbins et al. 2002 (mice) and Yano et al. 2002 (rats). The pathology and systemic toxicity noted in these studies are discussed in Section 3.1.5. Neither study found any evidence of carcinogenic activity. In addition, as summarized in several EPA risk assessments, spinosad was tested in a variety of standard *in vitro* and *in vivo* assays for mutagenicity, and mutagenic activity is not observed (U.S. EPA/OPP/HED 1997b, p. 12; U.S. EPA/OPP/HED 2009, p. 5; U.S. EPA/OPP/HED 2009, p. 3). Based on these data, the EPA concludes that spinosad is "*Not likely to be Carcinogenic to Humans*" (U.S. EPA/OPP/HED 2009a, Table A.2.1, P. 35).

Three studies on the mutagenic potential of spinosad are available in the literature from outside of the United States (Aciole et al. 2014 [Brazil]; Akmoutsou et al. 2011 [Greece]; El-Hoda et al. 2012 [Egypt]).

Increases in number of total structural aberrations in bone marrow chromosomes were observed in a subchronic study in rats (El-Hoda et al. 2012, Table 2 of study). As summarized in Appendix 1 (Table A1-2), this study used a Dow AgroSciences formulation of spinosad (Tracer®, 24% a.i., SC). The dietary concentrations of spinosad used in this study were 8 and 16 ppm. These concentrations are substantially below the concentrations used in the chronic toxicity study in rats (Bond et al. 1995b, MRIDs 43701507 and 43710503)—i.e., 50, 200, 500, or 1000 ppm. Given the lack of carcinogenic activity in the chronic study in rats as well as the supporting study in mice, the report from El-Hoda et al. (2012) does not substantially increase concern for the potential carcinogenicity of spinosad.

Exposure to spinosad (source and/or formulation not specified) resulted in an increase of mutations in the somatic mutation and recombination assay in *Drosophila melanogaster* at the highest concentration tested, 1.6 mg/L (Table 1 in Aciole et al. 2013). Using the same assay as Aciole et al. (2013) but at lower concentrations (0.1 to 0.5 mg a.i./L of a 480 g/L formulation of spinosad), Akmoutsou et al. (2011) observed no mutagenic activity in *Drosophila*. While the assays in *Drosophila* are noted for the sake of completeness, this type of *in vivo* assay in an insect does not raise concern for the carcinogenicity of spinosad, given the available chronic studies for carcinogenicity in rats and mice.

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

As with acute oral toxicity, the U.S. EPA/OPP requires acute assays for skin irritation, skin sensitization, and eye irritation and uses a ranking system for responses ranging from Category I (most severe response) to Category IV (least severe response) for skin and eye irritation. Skin sensitization is classified simply as occurring or not occurring. For each type of assay, the EPA has developed standard protocols (U.S. EPA/OCSPP 2013).

3.1.11.1. Skin Irritation

As summarized in Appendix 1 (Table A1-4), spinosad is not considered a skin irritant in rabbits (i.e., Category 4) based on assays of both technical grade spinosad (MRID 43414519) and a 44% a.i. formulation of spinosad (MRID 43414513). The only effect noted was slight and transient erythema (redness) and edema (NOS) in the assay conducted with the formulation. Consistent with the EPA assessment, regulatory reviews from Europe indicate that spinosyn is not regarded as a skin irritant (European Commission 2006; FAO/WHO 2011; WHO 2008, 2011).

The descriptions of skin irritation studies on the MSDS for the representative formulations (Table 4) range from non-irritating to slight irritation. These descriptions are consistent with the studies summarized in Appendix 1, Table A1-4.

In addition to the standard studies required by EPA for the use of spinosad as an insecticide, studies involving applications of spinosad to humans are available because spinosad is approved by the FDA for the treatment of head lice. Most studies and reviews covering this use in humans indicate a lack of adverse effects (Cole and Lundquist 2011; Gunning et al. 2012; Shmidt and Levitt 2012). In a large study involving 552 participants using a 0.9% solution of spinosad for the treatment of head lice, application site erythema was noted in 3.1% (n=17) of the participants and application site irritation was noted in 0.9% (n=4) of the participants (Stough et al. 2009, Table 2, p. e392). This study did not involve a control group, and the statistical significance of the reports of erythema and irritation is not clear. Stough et al. (2009, Table 2, p. e392) provide statistics based on Fischer's Exact Test; however, these statistics appear to apply to comparisons of spinosad with permethrin, another pesticide included in the study. Nonetheless, the erythema and irritation noted in the study by Stough et al. (2009) seems consistent with the mild dermal effects observed in rabbits treated with a formulation of spinosad (MRID 43414513).

While mild skin irritation might be noted in the handling of spinosad formulations, there is no basis for asserting that serious skin irritation or other skin damage is likely.

3.1.11.2. Skin Sensitization

The assay for skin sensitization in guinea pigs is a standard assay (U.S. EPA/OPPTS 2003). As summarized in Appendix 1 (Table A1-4), this standard assay was conducted on both technical grade spinosad (MRID 43414520) and a 44% a.i. formulation of spinosad (MRID 43414513). Both assays found no evidence of skin sensitization; accordingly, the EPA concludes that spinosad is not a skin sensitizer (U.S. EPA/OPP/HED 1997b, 2009a, 2010b, 2011a). This classification is consistent with literature from the European regulatory community (European Commission 2006; FAO/WHA 2001; WHO 2008, 2011). The MSDS/SDS for all of the representative formulations considered in the current risk assessment (Table 4) indicate that the formulation or a.i. does not cause skin sensitization.

As discussed in Section 3.1.11.1, spinosad is used to treat head lice. Reports of skin sensitization associated with this use are not reported in the available literature (Cole and Lundquist 2011; Gunning et al. 2012; Shmidt and Levitt 2012; Stough et al. 2009).

Given the standard assays in guinea pigs and the human experience with spinosad, there is no basis for identifying skin sensitization as an endpoint of concern.

3.1.11.3. Ocular Effects

As with skin irritation and skin sensitization, standard assays for eye irritation are available on both technical grade spinosad (MRID 43414518) and a 44% a.i. formulation of spinosad (MRID 43414512). These studies are summarized in Appendix 1 (Table A1-5). Slight conjunctival irritation was observed in both studies. Based on the minimal responses in these studies, U.S. EPA/OPP/HED categorizes both technical grade spinosad and the formulation of spinosad as Category IV (i.e., the least severe category) for eye irritation (U.S. EPA/OPP/HED 1997b, 2009a, 2010b, 2011a).

Most of the descriptions of eye irritation studies on the MSDS for the representative formulations (Table 4) are consistent with the studies summarized in Appendix 1, Table A1-5. The one possible exception is Entrust [WP]. The SDS for this formulation indicates that the formulation…*Causes serious eye irritation.*

The European Commission (2006) states that spinosad is not irritating to the eyes. Consistent with the EPA assessment, other regulatory reviews of spinosad note that spinosad is a slight eye irritant (FAO/WHO 2001; WHO 2008, 2011).

As discussed in Section 3.1.11.1, Stough et al. (2009) published a large study on the use of a 0.9% formulation of spinosad to treat head lice in humans. Of the 552 participants in this study treated with spinosad, 12 (2.2%) reported *ocular hyperemia*—i.e., redness or inflammation of the eyes (Stough et al. 2009, Table 2). This effect is noted but is not otherwise discussed in the study. As also discussed in Section 3.1.11.1, this study did not involve a control group and it is not clear if the response noted by Stough et al. (2009) was statistically significant. Stough et al. (2009, Table 2, p. e392) provide statistics based on Fischer's Exact Test; however, these statistics appear to apply to comparisons of spinosad with permethrin, another pesticide included in the study. For ocular hyperemia, the reported *p*-value (0.329) would not be viewed as statistically significant.

Based on the available information, minimal eye irritation might be associated with exposures to spinosad; however, there is no basis for asserting that serious eye damage would be likely.

3.1.12. Systemic Toxic Effects from Dermal Exposure

The acute and repeated dose dermal toxicity studies on spinosad and spinosad formulations are summarized in Appendix 1, Table A1-6. The acute EPA studies (MRID 43414516 and MRID 43557503) as well as an acute study summarized by FAO/WHO (2001) are consistent indicating no observed adverse effects at doses of up to 2000 mg/kg bw in the EPA studies and 5000 mg/kg bw in the study summarized by EPA. The EPA studies were used to classify spinosad as Category IV (i.e., the least severe category) for acute dermal toxicity (U.S. EPA/OPP/HED 2011a, p. 5). All of the representative formulations explicitly covered in the current risk

assessment indicate dermal LD_{50} values in rabbits of >5000 mg/kg bw. These statements are consistent with the data presented in FAO/WHO 2011 (citing Stebbins and Brooks 1999a) but are somewhat higher than the values of >2000 mg/kg bw/day cited in EPA documents (Appendix 1, Table A1-6).

The repeated dose dermal studies contain inconsistencies. Study summaries provided in U.S. EPA/OPP/HED (1992b, 2009a) and FAO/WHO (2001) indicate no adverse effects in rabbits at doses of up to 1000 mg/kg bw/day with exposure periods of 6 hours/day for 21 days. An EPA compendia of DERs (U.S. EPA-OPP-HED 1997a), however, contains a DER for the study by Vedula and Yano (1994) in which a 43.4% formulation was assayed in rabbits at doses of 0, 100, 500, or 1000 mg/kg bw/day in a Phase 1 study and 0, 200, 300, or 500 mg/kg bw/day in a Phase 2 study. In both studies, the exposures consisted of 6 hours/day for 21-days with occlusion at the application sites to minimize potential ingestion of the test compound. While no frank signs of toxicity were noted, hyperplasia of the gastric mucosa was observed at doses of 300, 400, 500, or 1000 mg/kg bw/day. Although the responses at 300 mg/kg bw/day were not statistically significant, the responses at 400, 500, and 1000 mg/kg bw/day were statistically significant using the Fischer's Exact Test. It should be noted one study from (FAO/WHO 2001) is cited as Vedula and Yano (1994) with a study number of DR-0323-1194-018. This study is not identical to the DER of Vedula and Yano (1994) which has study numbers of DR-0341-0784-002 and DR-0341-0784-002R. It should also be noted that the EPA human health risk assessments (U.S. EPA/OPP/HED 1997b, 2009a, 2010b, 2011a) do not include a discussion of Vedula and Yano (1994).

The above discrepancies are noted only for the sake of completeness. As discussed in Section 3.1.3.2.1, U.S. EPA/OPP/HED does not explicitly consider dermal exposures in their risk assessments for spinosad, in part, due to the lack of toxicity observed in the repeated dose dermal studies. While the above discrepancies do not have a substantial impact on this decision, the current risk assessment does explicitly and quantitatively consider dermal exposures for both workers and members of the general public (Section 3.2).

3.1.13. Inhalation Exposure

The U.S. EPA typically requires short-term (single 4-hour exposure) inhalation toxicity studies in rats (U.S. EPA/OPPTS 1998b) to support pesticide registration. As summarized in Appendix 1 (Table A1-7), these standard studies are available for technical grade spinosad (MRID 43414517) as well as an unspecified 44% a.i. formulation of spinosad (MRID 43414511). The EPA may sometimes require subchronic inhalation studies (U.S. EPA/OPPTS 1998c), but these studies have not been required for spinosad.

The EPA documents report indefinite acute LC_{50} values of >5.18 mg/L for technical grade spinosad (MRID 43414517) and >5 mg/L for an unspecified 44% a.i. formulation (MRID 43414511). As noted in Table 2, the spinosyns have low vapor pressures – i.e., 2 to 3×10^{-5} mPa at 25 °C. While details of the inhalation toxicity studies cited by EPA are not available, concentrations in the range of 5 mg/L are not attainable in vapor form but must involve aerosols.

As summarized in Table 4, MSDS for most of the representative formulations explicitly covered in the current risk assessment report LC_{50} values over a similar range—i.e., from >4.19 to >5.51 mg/L. The SDS for Conserve SC reports a higher indefinite LC_{50} of >17.02 mg/L. This value,

however, is specified on the SDS as applying to the formulation (11.6% a.i.). Thus, the LC_{50} corresponds to about >2 mg a.i./L [17.02 mg/L x 0.116 = 1.97432]. Since all of these LC_{50} values are indefinite, the differences in the values simply reflect differences in the experimental concentration(s) used in the assays and do not necessarily reflect any differences in potency among the different formulations.

Based on the available inhalation bioassays, the EPA classifies spinosad at Category IV for acute inhalation exposure—i.e., the least hazardous ranking (U.S. EPA/OPP/HED 2011a, p. 5). As discussed further in Section 3.2.2 (worker exposure), the U.S. EPA explicitly considers inhalation as a route of concern for occupational exposures in their more recent risk assessments (e.g., U.S. EPA/OPP/HED 2009a). This approach differs from the determination in earlier EPA risk assessments that…*Exposure via inhalation is not a concern* (U.S. EPA/OPP/HED 1997b, p. 14). As with most Forest Service risk assessments, the occupational exposure assessments for workers in the current risk assessment are based on biomonitoring studies which implicitly consider all routes of exposure (i.e., dermal, inhalation, and incidental oral routes).

3.1.14. Adjuvants and Other Ingredients

3.1.14.1. Other Ingredients

The U.S. EPA is responsible for regulating both the active ingredients (a.i.) in pesticide formulations as well as any other chemicals that may be added to the formulation. As implemented, these regulations affect only pesticide labeling and testing requirements. The term *inert* was used to designate compounds that are not classified as active ingredient on the product label. While the term *inert* is codified in FIFRA, some inerts can be toxic, and the U.S. EPA now uses the term *Other Ingredients* rather than *inerts* (http://www.epa.gov/opprd001/inerts/). For brevity, the following discussion uses the term *inert*, recognizing that *inerts* may be biologically active and potentially hazardous components.

The identities of inerts in pesticide formulations are generally considered trade secrets and need not be disclosed to the general public. Nonetheless, all inert ingredients as well as the amounts of the inerts in the formulations are disclosed to and reviewed by the U.S. EPA as part of the registration process. Some inerts are considered potentially hazardous and are identified as such on various lists developed by the federal government and state governments. Material Safety Data Sheets or Safety Data Sheets sometimes specify inerts used in pesticide formulations. U.S. EPA/OPP (2015b, p. 5-13) encourages but does not generally require expanded inert statements on product labels which specifically identify the inert ingredients in the product. One notable exception, however, involves petroleum distillates including xylene or xylene range solvents that are part of the formulation and at a concentration of ≥10%. In this case, the product label must contain the following statement: *Contains petroleum distillates, xylene or xylene range aromatic solvents* (U.S. EPA/OPP 2010d, p. 5-11). None of the product labels for the representative formulations listed in Table 3 indicates that these formulations contain petroleum distillates at or above 10% of the formulation.

The U.S. EPA classifies inerts into one of four lists based on the available toxicity information: toxic (List 1), potentially toxic (List 2), unclassifiable (List 3), and non-toxic (List 4). List 4 is subdivided into two categories, 4A and 4B. List 4A constitutes inerts for which there is adequate information to indicate a minimal concern. List 4B constitutes inerts for which the use patterns

and toxicity data indicate that use of the compound as an inert is not likely to pose a risk. These lists as well as other updated information regarding pesticide inerts are maintained by the U.S. EPA at the following web site: http://www.epa.gov/opprd001/inerts/.

As summarized in Table 3, the inerts specified on the MSDS/SDS for the representative formulations explicitly considered in the current risk assessment include propylene glycol, kaolin, and silica gel. Several of the formulations in Table 3 indicate that the formulations contain propylene glycol. Propylene glycol is a List 4B inerts and is exempt from tolerances as a food-use inert ingredient under the Code of Federal Regulations (40 CFR part 180). Kaolin (1332-58-7) is a form of clay. Silica gel and clay are categorized at a List 4A inerts—i.e., inerts of minimal concern.

For all of the formulations listed in Table 3, the percentage of spinosad combined with the percentages of inerts do not total to 100%. In other words, there are unspecified inerts in all of the formulations. Nonetheless, as noted above, all inerts are disclosed to and approved by the U.S. EPA. One inert often not listed on MSDS/SDS is water. While speculative, it seems reasonable to suggest that at least some of the proportion of undisclosed inerts in the formulations listed in Table 3 may consist of water.

3.1.14.2. Adjuvants

As with most Forest Service risk assessments as well as pesticide risk assessments conducted by the EPA, the current risk assessment does not specifically attempt to assess the risks of using adjuvants, without specific information to suggest that the risks may be substantial. For example, some adjuvants used in glyphosate formulations may be as toxic as, and possibly more toxic than, glyphosate itself; accordingly, these risks are addressed in the Forest Service risk assessment on glyphosate (SERA 2010). Comparable information is not available on adjuvants that might be used with spinosad.

The product labels for representative formulations of spinosad (Table 3) indicate that emulsified or methylated crop oil as well as organosilicone surfactants may be used in applications for the control of some pests, however, fuel oil and mineral oil should not be used.

3.1.15. Impurities and Metabolites

3.1.15.1. Metabolites

As discussed in SERA (2014a, Sections 3.1.3.1), two types of metabolites may be considered in a risk assessment, *in vivo* metabolites and environmental metabolites. *In vivo* metabolites refer to the compounds formed within the animal after the pesticide has been absorbed. Environmental metabolites refer to compounds that may be formed in the environment by a number of different biological or chemical processes, including breakdown in soil or water or breakdown by sunlight (photolysis).

The *in vivo* metabolites of spinosad are discussed in Section 3.1.3.1, and an overview of these metabolites is given in Figure 4. As illustrated in Figure 4, the major mammalian *in vivo* metabolites involve N- and O-demethylation as well as conjugation with glutathione. The environmental metabolism of spinosad is reviewed in detail by Cleveland et al. (2002a, see Figure 1 of their paper) and Mandal et al. (2013, see Figure 2 of their paper). The environmental

metabolism of spinosad is also covered in some detail in various EPA risk assessments (as cited in Section 1), particularly U.S. EPA/OPP/HED (2009a, Section 4.1). As with *in vivo* mammalian metabolism, environmental metabolites are formed through N- and O-demethylation, leading to several different metabolites that are structurally similar to spinosyns A and D (Figure 1 of this risk assessment). Additional environmental metabolites are formed by cleavage of the forosamine sugar and/or the rhamnose ring, reductions in the macrolide ring, hydrolysis, and dehydroxylation (Cleveland et al. 2002a; Mandal et al. 2013).

From a practical perspective, metabolites have an impact on the risk assessment when they are of comparable or greater toxicity than the parent compound. For spinosad, there is no indication that the metabolites are much more toxic to mammals than the parent compounds (spinosyns A and D). As discussed further in Section 4.1.3.3, this is not the case for aquatic invertebrates, which does not have an impact on the hazard identification for human health effects. No information is available on the toxicity of the environmental metabolites to humans or experimental mammals. Noting the structural similarity of most metabolites of spinosad to the parent compounds, U.S. EPA/OPP/HED (2011a, p. 9) adopts a total residue approach for aquatic modeling. Essentially, this method assumes that the toxicities of the metabolites are comparable to the toxicities of the parent compounds. This approach is also adopted in the current risk assessment, as detailed further in Section 3.2.3 (exposure assessments for members of the general public).

3.1.15.2. Impurities

Information on the impurities in spinosad is not available in the published literature (Table 1) or the EPA documents on spinosad (listed in Section 1). As discussed in Section 2, spinosad is formed in fermentation by the *Saccharopolyspora spinosa*. Thus, it seems reasonable to assume that some impurities may occur in technical grade spinosad. As summarized in Appendix 1 as well as other appendices to this risk assessment, the purity of technical grade spinosad is typically characterized as about 80 to 96%. The remainder of the material may be viewed as impurities.

Registrants disclose the nature of impurities in their technical grade material to the U.S. EPA; however, the identities of the impurities are not disclosed to the public, because that information may provide insight into the manufacturing process, which is considered proprietary and is protected under FIFRA (Section 10). Proprietary information on the identities of these impurities was not available for the preparation of the current Forest Service risk assessment.

To some extent, concern for impurities in technical grade spinosad is reduced because most of the existing toxicity studies were conducted with the technical grade product. Thus, any toxic impurities present in the technical grade product are likely to be encompassed by the available toxicity studies on the technical grade product.

3.1.16. Toxicologic Interactions

The only studies on toxicological interactions associated with spinosad are from the veterinary literature. As noted in Section 3.1.2, spinosad may inhibit P-glycoprotein, an ATP-dependent efflux pump involved in the inhibition in the uptake and active secretion of xenobiotics from cells (Ambudkar et al. 2003). Schrickx (2014) indicates that spinosad is a potent inhibitor of canine P-glycoprotein (i.e., IC_{50} of about 0.27 µM or 0.2 µg/mL). Dunn et al. (2011) suggests

that P-glycoprotein inhibition by spinosad is associated with the increased risk of ivermectin induced neurotoxicity in dogs. This interpretation, however, has been challenged by MacKay et al. (2012).

In studies on the joint action of spinosad and milbemycin oxime, both of which are used to treat fleas in dogs, Holstrom et al. (2012) note that co-exposure to spinosad increases systemic levels of milbemycin oxime. While not providing detailed experimental data, these investigators suggest that this interaction may be due to decreased metabolism of milbemycin oxime by cytochrome P450. Cytochrome P-450 is a general term for a class of mixed function oxidases involved in the metabolism of a broad range of naturally occurring chemicals (e.g., steroids) as well as xenobiotics (i.e., man-made chemicals typically not found in nature). In general, any compound that inhibits a mixed function oxidase may inhibit or alter the metabolism of other compounds that also serve as substrates for the mixed function oxidase. Furthermore, substrates for mixed function oxidases can often induce the production of mixed function oxidases, thereby enhancing their own metabolism as well as that of other compounds (e.g., Coon 2005; Lewis et al. 1998). Depending on the compounds involved in these interactions, the toxicity of the compounds could be enhanced (if P-450 detoxifies the compounds) or reduced (if P-450 metabolizes the compounds to more toxic metabolites). As discussed further in Section 4.1.2.4.1, the available information on insects clearly indicates that metabolism by cytochrome P-450 is a detoxification mechanism in terrestrial insects. Comparable data in mammals has not been identified.

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview

The exposure assessments used in the current risk assessment are given in the accompanying EXCEL workbooks: Attachment 1 for a single application and Attachment 2 for two applications with a 6-day application interval. These workbooks contain a set of worksheets that detail each exposure scenario discussed in this risk assessment as well as summary worksheets for both workers (Worksheet E01) and members of the general public (Worksheet E02). Documentation for these worksheets is presented in SERA (2011a). All exposure assessments are conducted assuming an application rate of 0.225 lb a.i./acre (Section 2).

Worker exposures are modeled for backpack spray, broadcast ground spray, and aerial spray. In non-accidental scenarios involving the normal application of spinosad, central estimates of exposure for workers are approximately 0.000015 mg/kg/day for backpack applications, 0.00008 mg/kg/day for ground broadcast applications, and 0.000007 mg/kg bw/day for aerial spray. Estimates of upper bound exposures are approximately 0.0002 mg/kg/day for backpack applications, 0.006 mg/kg/day for ground broadcast applications, and 0.004 mg/kg/day for aerial applications. As discussed further in Section 3.4, these exposure estimates are far below the level of concern, reflecting the poor dermal absorption of spinosad. Because all worker exposure estimates used in Forest Service risk assessments assume that the worker applies the pesticide over an application season, the worker exposures for both one and two applications at a single site are identical. In other words, the worker is assumed to apply the pesticide repeatedly over the course of the application season. Whether this is done at a single site or multiple sites is incidental to the exposure.

For the general public (Worksheet E03), acute non-accidental exposure levels associated with a single application range from very low (e.g., $\approx 3.5 \times 10^{-7}$ mg/kg/day) to about 0.3 mg/kg bw. Because of the persistence of spinosad and the relatively brief application interval, most of the estimated doses for two applications are about twice as high as those for a single application. As with most exposure assessments involving foliar applications, the highest levels of exposure are associated with the consumption of contaminated vegetation (i.e., upper bound doses of up to about 0.3 mg/kg bw/day for a single application and 0.6 mg/kg bw/day for two applications). The lowest exposure levels are associated with swimming in contaminated water (i.e., upper bound doses of about 1×10^{-6} mg/kg bw/day for a single application and 2×10^{-6} mg/kg bw/day for two applications). For the accidental exposure scenarios, the greatest exposure levels are associated with the consumption of contaminated water by a small child following an accidental spill, for which the upper bound dose is about 0.5 mg/kg bw. The accidental exposure scenarios for the general public are identical for both one and two applications because these scenarios involve only a single accidental event.

3.2.2. Workers

3.2.2.1. General Exposures

All general exposures for workers are calculated as the amount a.i. handled by a worker in a single day multiplied by a worker exposure rate (in units of mg/kg bw per lb a.i. handled). Relatively well-documented worker exposure rates are available (SERA 2014b) for bark applications as well as foliar broadcast applications.

In Table 14 of SERA (2014b), three reference chemicals with corresponding worker exposure rates are given for directed foliar applications with differing first-order dermal absorption rate coefficients ($k_a$values)—i.e., glyphosate (k_a = 0.00041 hour^{-1}), 2,4-D (k_a = 0.00066 hour^{-1}), and triclopyr BEE (k_a = 0.0031 hour^{-1}). As discussed in Section 3.1.3.2.2 of the current risk assessment, the central estimate of the first-order dermal absorption rate coefficient for spinosad is 0.00002 hour^{-1}. This rate coefficient for spinosad is about a factor of about 20 less than the corresponding coefficient for glyphosate, the reference pesticide with the lowest k_a [0.00041 hour^{-1} ÷ 0.00002 hour^{-1} = 20.5]. While a factor of 20 involves substantial extrapolation, glyphosate is used as the reference chemical for directed foliar applications in order to minimize extrapolation. For directed foliar applications, the application of the methodology from SERA (2014b) is detailed in Table 5. The rates given in Table 5 are rounded to two significant digits and are used in Worksheet C01a the attachments to the risk assessment to estimate exposures for workers involved in directed foliar applications.

As also summarized in Table 14 of SERA (2014b), only one reference chemical, 2,4-D, is available for ground broadcast and aerial applications, and the first-order dermal absorption rate coefficient for 2,4-D is taken as 0.00066 hour^{-1}. This first-order dermal absorption rate coefficient is below the corresponding value for spinosad by a factor of over 30 [0.00066 hour^{1} ÷ 0.00002 hour^{-1} ≈ 33.0033]. While the application of dermal adjustment factors is optional in the SERA (2014b) methodology, the dermal adjustment factor is used in this risk assessment of spinosad. The most recent EPA occupational exposure assessments do not specifically consider dermal absorption (U.S. EPA/OPP/HED 2009a, Table 7.1.1, p. 37). As noted in Section 3.1.3.2.1 (First-Order Dermal Absorption), not addressing dermal absorption reflects the EPA's assessment of the poor dermal absorption and low dermal toxicity of spinosad. Given the approach taken by EPA and for the same reasons—i.e., apparent poor dermal absorption and low dermal toxicity—the application of the dermal absorption adjustment factor for spinosad seems reasonable. These adjustments are detailed in Table 8 (ground broadcast applications) and Table 9 (aerial applications).

In addition to the application rate and absorbed dose rate, the other factor affecting worker exposure is the number of acres per day that a worker will treat, in that acres treated per day are used in estimating the amount of pesticide that a worker will handle. Estimates of the number of acres per day that a worker might treat are taken from SERA (2014b, Table 2 and Section 1.1). These estimates are as important as worker exposure rates, and estimates of the number of acres treated per day should be adjusted as appropriate for any site-specific application.

3.2.2.2. Accidental Exposures

Generally, dermal exposure is the predominant route of exposure for pesticide applicators (Ecobichon 1998; van Hemmen 1992); hence, accidental dermal exposures are considered quantitatively in all Forest Service risk assessments. The two types of dermal exposures modeled in the risk assessments include direct contact with a pesticide solution and accidental spills of the pesticide onto the surface of the skin. In addition, 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 EXCEL workbooks that accompany this risk

assessment—i.e., Attachments 1 and 2. Additionally, Worksheet E01 references other worksheets in which the calculations of each exposure assessment are detailed.

Exposure scenarios involving direct contact with solutions of spinosad are characterized either by immersion of the hands in a field solution for 1 minute or wearing pesticide contaminated gloves for 1 hour. The assumption that the hands or any other part of a worker's body will be immersed in a chemical solution for a prolonged period of time may seem unreasonable; however, it is possible that the gloves or other articles of clothing worn by a worker may become contaminated with pesticide resulting in potentially long periods of exposure. For these exposure scenarios, the key assumption is that wearing gloves grossly contaminated with a chemical solution is equivalent to immersing the hands in the solution. In both cases, the chemical concentration in contact with the skin and the resulting dermal absorption rate are essentially constant. For both scenarios (hand immersion and contaminated gloves), the assumption of zero-order absorption kinetics is appropriate. For these types of exposures, the rate of absorption is estimated based on a zero-order dermal absorption rate (K_p). Details regarding the derivation of the K_p value for spinosad are provided in Section 3.1.3.2.2. The amount of the pesticide absorbed per unit time depends entirely on the concentration of the chemical in solution. This concentration is highly variable depending on the application method and also on the dilution volumes, as discussed in Section 2.4. These exposure scenarios are detailed in Worksheets C02a (1-minute exposure) and C02b (60-minute exposure).

The details of the accidental spill scenarios for workers consist of spilling a chemical solution on to the lower legs as well as spilling a chemical solution on to the hands, at least some of which adheres to the skin. The absorbed dose is then calculated as the product of the amount of chemical on the skin surface (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 chemical concentration in the liquid), the first-order absorption rate coefficient, and the duration of exposure. The first-order dermal absorption rate coefficient (k_a) is derived in Section 3.1.3.2.1. These exposure scenarios are detailed in Worksheets C03a (spill on to the hand) and C03b (spill onto the lower legs).

3.2.3. General Public

3.2.3.1. General Considerations

3.2.3.1.1. Likelihood and Magnitude of Exposure

The likelihood that members of the general public will be exposed to spinosad in Forest Service programs appears to be highly variable, depending on which of the various application methods is used and the sites at which spinosad might be applied. Spinosad could be applied in or near recreational areas like campgrounds, picnic areas, and trails. Under such circumstances, it is plausible that members of the general public would be exposed to spinosad, particularly in broadcast applications. Conversely, members of the general public are less likely to be exposed to spinosad if the pesticide is applied in remote areas.

Because of the conservative exposure assumptions used in the current risk assessment, neither the probability of exposure nor the number of individuals who might be exposed has a substantial impact on the characterization of risk presented in Section 3.4. As detailed in SERA (2014a, Section 1.2.2.2), the exposure assessments developed in this risk assessment are based

on *Extreme Values* rather than a single value. Extreme value exposure assessments, as the name implies, bracket the most plausible estimate of exposure (referred to statistically as the central or maximum likelihood estimate and more generally as the typical exposure estimate) with extreme lower and upper bounds of plausible exposures.

This Extreme Value approach is essentially an elaboration on the concept of the *Most Exposed Individual* (MEI), sometime referred to as the *Maximum Exposed Individual* (MEI). As this name also implies, exposure assessments that use the MEI approach are made in an attempt to characterize the extreme but still plausible upper bound on exposure. This approach is common in exposure assessments made by U. S. EPA, other government agencies, and other organizations. In the current risk assessment and other Forest Service risk assessments, the upper bounds on exposure estimates are all based on the MEI.

In addition to this upper bound MEI value, the Extreme Value approach used in this risk assessment provides a central estimate of exposure as well as a lower bound on exposure. While not germane to the assessment of upper bound risk, it is significant that the use of the central estimate and especially the lower bound estimate is not intended to lessen concern. To the contrary, the central and lower estimates of exposure are used to assess the feasibility of mitigation—e.g., protective measures to limit exposure. If lower bound exposure estimates exceed a level of concern, this is strong indication that the pesticide cannot be used in a manner that will lead to acceptable risk.

3.2.3.1.2. Summary of Assessments

The exposure scenarios developed for the general public are summarized in Worksheet E03 of the EXCEL workbooks that accompany this risk assessment. As with the worker exposure scenarios, details about the assumptions and calculations used in these assessments are given in the detailed calculation worksheets in the EXCEL workbooks (Worksheets D01–D10).

For spinosad, a standard set of exposure assessments used in all Forest Service risk assessments for broadcast applications is considered. As summarized in Worksheet E03 of Attachments 1 and 2, the kinds of exposure scenarios developed for the general public include acute accidental, acute non-accidental, and longer-term or chronic exposures. The accidental exposure scenarios assume that an individual is exposed to the compound of concern either during or shortly after its application. Non-accidental exposures involve dermal contact with contaminated vegetation as well as the consumption of contaminated fruit, vegetation, water, or fish. The longer-term or chronic exposure scenarios parallel the acute exposure scenarios for the consumption of contaminated fruit, water, or fish. All of the non-accidental exposure scenarios are based on levels of exposure to be expected following an application of spinosad at 0.225 lb a.i./acre (Attachment 1) or two applications at 0.225 lb a.i./acre with a 6-day application interval (Attachment 2). The upper bounds of the exposure estimates for the non-accidental scenarios involve conservative assumptions intended to reflect exposure for the MEI (*Most Exposed Individual*). The impact on the risk characterization of lower application rates is discussed in Section 3.4.

The nature of the accidental exposure scenarios is intentionally extreme. The non-accidental, acute exposure scenarios are intended to be conservative but plausible, meaning that it is not unreasonable to assume that the magnitude of exposures in the non-accidental exposure scenarios

could occur in the routine use of spinosad. This interpretation does not extend to the longer-term exposure scenarios. The longer-term exposure scenarios essentially assume that an individual will consume either contaminated vegetation, fruits, or water from a treated area every day over a prolonged period of time. However unlikely it may seem, this type of exposure cannot be ruled out completely. As discussed further in Section 3.4.3, this is an important consideration in the interpretation of hazard quotients associated with longer-term exposures to contaminated vegetation.

3.2.3.2. Direct Spray

Direct spray scenarios for members of the general public 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 field solution of the compound and that some amount of the compound remains on the skin and is absorbed by first-order kinetics. Two direct spray scenarios are given, one for a young child (D01a) and the other for a young woman (D01b).

For the young child, it is assumed that a naked child is sprayed directly during a broadcast application and that the child is completely covered with pesticide (i.e., 100% of the surface area of the body is exposed). This exposure scenario is intentionally extreme. As discussed in Section 3.2.3.1.1, the upper limits of this exposure scenario are intended to represent the *Extreme Value* of exposure for the *Most Exposed Individual* (MEI).

The exposure scenario involving the young woman (Worksheet D01b) is somewhat less extreme, but more plausible, and assumes that the woman is accidentally sprayed over the feet and lower legs. By reason of allometric relationships between body size and dose-scaling, a young woman would typically be subject to a somewhat higher dose than would the standard 70 kg man. Consequently, in an effort to ensure a conservative estimate of exposure, a young woman, rather than an adult male, is used in many of the exposure assessments.

For the direct spray scenarios, assumptions are made regarding the surface area of the skin and the body weight of the individual, as detailed in Worksheet A03 of the attachments. The rationale for and sources of the specific values used in these and other exposure scenarios are provided in the documentation for WorksheetMaker (SERA 2011a) and in the methods document for preparing Forest Service risk assessments (SERA 2014a).

3.2.3.3. Dermal Exposure from Contaminated Vegetation

In this exposure scenario, it is assumed that spinosad is sprayed on to vegetation and that a young woman comes in contact with sprayed vegetation or other contaminated surfaces at some period after the spray operation (D02). For these exposure scenarios, some estimates of dislodgeable residue (a measure of the amount of the chemical that could be freed from the vegetation) and the rate of transfer of the chemical from the contaminated vegetation to the surface of the skin must be available.

No data are available on dermal transfer rates for spinosad. This is not a severe limitation in this risk assessment. As detailed in Durkin et al. (1995), dermal transfer rates are reasonably consistent for numerous pesticides, and the methods and rates derived in Durkin et al. (1995) are used as defined in Worksheet D02. Similarly, no data are available on dislodgeable residues for spinosad. Again citing the low dermal toxicity of spinosad, U.S. EPA/OPP (2015c, p. 80669) indicates that …*dislodgeable-foliar residue (DFR) studies are unnecessary at this time as there is no hazard via the dermal route of exposure*. In the absence of data, a default dislodgeable residue rate of 0.1 of the nominal application rate is used for this exposure scenario.

The exposure scenario assumes a contact period of 1 hour and further assumes that the chemical is not effectively removed by washing for 24 hours. Other approximations used in this exposure scenario include estimates of body weight, skin surface area, and first-order dermal absorption rates, as discussed in Section 3.2.3.2 (Direct Spray).

3.2.3.4. Contaminated Water

3.2.3.4.1. Accidental Spill

The accidental spill scenario assumes that a young child consumes contaminated water shortly after an accidental spill of a field solution into a small pond. The calculation of the concentration of spinosad in water following the spill is given in Worksheet B04b, and the estimate of the dose to a small child is given in Worksheet D05 of the attachments to this risk assessment. Because this scenario is based on the assumption that exposure occurs shortly after the spill, no dissipation or degradation is considered.

Since this exposure scenario is based on assumptions that are somewhat subjective and highly variable, the scenario may overestimate exposure. The actual chemical concentrations in the water will vary according to 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. All Forest Service risk assessments assume that the accidental spill occurs in a small pond with a surface area of about one-quarter of an acre (1000 m^2) and a depth of 1 meter. Thus, the volume of the pond is 1000 m^3 or 1,000,000 liters.

For applications of spinosad, a spill volume of 100 gallons with a range of 20 to 200 gallons is used to reflect plausible spill events. These spill volumes are used in all Forest Service risk assessments involving terrestrial applications of liquid applications. The spinosad concentrations in the field solution are also varied to reflect the plausible range of concentrations in field solutions—i.e., the material that might be spilled—using the same values as in the accidental exposure scenarios for workers (Section 3.2.2.2). Based on these assumptions, the estimated nominal concentration of spinosad in a small pond ranges from about 0.1 to about 4 mg/L with a central estimate of about 1 mg/L.

3.2.3.4.2. Accidental Direct Spray/drift for a Pond or Stream

These scenarios involve the accidental direct spray or incidental spray drift to a small pond and a small stream. The exposure scenarios involving drift are less severe but more plausible than the accidental spill scenario described in the previous section. The drift estimates are based on AgDrift, as detailed in SERA (2011b, Section 3.3.2). The direct spray and drift scenarios are

detailed in Worksheet B04c (small pond) and Worksheet B04d (small stream). As would be expected, the concentrations for direct spray are far below the concentrations associated with the accidental spill—i.e., about 0.025 mg/L for a small pond and 0.02 mg/L for a small stream. Also, as expected, the concentrations associated with drift are much lower. Using a distance of 25 feet down wind as examples, the concentrations in a small pond and a small stream are about 0.0002 to 0.004 mg/L, depending on the application method.

3.2.3.4.3. GLEAMS Modeling

The Forest Service developed a program, Gleams-Driver, to estimate expected peak and longer-term pesticide concentrations in surface water. Gleams-Driver serves as a preprocessor and postprocessor for GLEAMS (Knisel and Davis 2000). GLEAMS is a field scale model developed by the USDA/ARS and has been used for many years in Forest Service and other USDA risk assessments (SERA 2007a, 2011b).

Gleams-Driver offers the option of conducting exposure assessments using site-specific weather files from Cligen, a climate generator program developed and maintained by the USDA Agricultural Research Service (USDA/NSERL 2004). Gleams-Driver was used in the current risk assessment to model spinosad concentrations in a small stream and a small pond.

As summarized in Table 10, nine locations are used in the Gleams-Driver modeling. These locations are standard sites used in Forest Service risk assessments for Gleams-Driver simulations and are intended to represent combinations of precipitation (dry, average, and wet) and temperature (hot, temperate, and cool) (SERA 2007a). The characteristics of the fields and bodies of water used in the simulations are summarized in Table 11. For each location, simulations were conducted using clay (high runoff, low leaching potential), loam (moderate runoff and leaching potential), and sand (low runoff, high leaching potential) soil textures. For each combination of location and soil, Gleams-Driver was used to simulate pesticide losses to surface water from 100 modeled applications at a unit application rate of 1 lb a.i./acre, and each of the simulations was followed for a period of about 1½ years post application. Note that an application rate of 1 lb a.i./acre is used as a convention in all Forest Service risk assessments in order to avoid rounding limitations in GLEAMS outputs and are referred to as water contamination rates (WCR), concentrations in water associated with an application rate of 1 lb/acre. In the workbooks that accompany this risk assessment, the WCRs are converted to expected concentrations by multiplying the WCRs by the anticipated application rate of 0.225 lb a.i./acre as discussed in Section 2 (Program Description). As also discussed in Section 2, separate simulations are run for a single application (Appendix 8) and two applications with an application interval of 6 days (Appendix 9).

Table 12 summarizes the chemical-specific values used in Gleams-Driver simulations. For the most part, the chemical properties used in the Gleams-Driver simulations are based on the parameters used by the Environmental Fate and Effects Division (EFED) of the U.S. EPA's Office of Pesticides Programs modeling of spinosad (U.S. EPA/OPP/EFED 2009a). One substantial difference between the EPA and GLEAMS-Driver modeling involves estimates of variability. The EPA modeling is typically based on either central estimates or upper bound (90^{th} percentile) input parameters. Following the Extreme Value approach discussed in Section 3.2.3.1.1, the input parameters for the GLEAMS-Driver modeling are based on estimates of variability either as ranges or confidence intervals when estimates of variability are available.

For spinosad, the estimates of variability are made for foliar half-life, soil binding (K_{oc}), and sediment binding (K_d). In the GLEAMS-Driver simulations, the central estimates with lower and upper bounds are implemented as triangular distributions (SERA 2007a). In the current risk assessment, most of the model input values are based on the environmental fate studies submitted to the U.S. EPA by registrants, standard values for GLEAMS modeling recommended by Knisel and Davis (2000), and studies from the open literature. The notes to Table 10 indicate the specific sources of the chemical properties used in the GLEAMS modeling effort. The most substantial deviations of inputs used in the current risk assessment from the modeling inputs used by EPA include estimates of the variability in soil and sediment binding (K_{oc} and K_d values). Another difference between the EPA and GLEAMS-Driver runs involves half-lives in aquatic sediment, soil, and water. As discussed in Section 3.1.15.1, U.S. EPA/OPP/HED adopted a total residue approach for aquatic modeling of spinosad, because the metabolites are assumed to be comparable in toxicity to the parent compounds (U.S. EPA/OPP/HED 2011a, p. 9). As discussed in Section 3.2.3.4.4, some EPA models accommodate a zero degradation rate. This is not the case with GLEAMS which requires half-lives rather than degradation rates. Consequently, the GLEAMS-Driver inputs for half-lives in aquatic sediment, soil, and water are each set at 7,300 days (i.e., about 20 years).

Table 13 summarizes the modeled concentrations of spinosad in surface water by GLEAMS-Driver. Details of the GLEAMS-Driver simulations are detailed in Appendix 7 for a single application and Appendix 8 two applications with a 6-day application interval. Note that the concentrations modeled for two applications with an application interval of 6 days are approximately twice those of a single application. This relationship follows from the essential stability of spinosad and spinosad metabolites as discussed above. The specific concentrations of spinosad in surface water used in the exposure assessments for the current risk assessment are discussed in Section 3.2.3.4.6, following a comparison of the GLEAMS-Driver simulations with surface water models used by EPA (Section 3.2.3.4.4).

3.2.3.4.4. Other Modeling Efforts

Along with the GLEAMS-Driver modeling, Table 13 summarizes the results of the application of two EPA Tier 1 screening models to estimating concentrations of spinosad in surface water (FIRST) and ground water (PRZM-GW). The inputs and outputs for these Tier 1 models are detailed in Appendix 10. Table 11 also summarizes the EPA application of PRZM/EXAMS, a Tier 2 model (U.S. EPA/OPP/EFED 2005). The U.S. EPA/OPP typically models pesticide concentrations in water at the maximum labeled rate. In Table 13, the modeling results reported in U.S. EPA/OPP/EFED (2005a, p. 33) are normalized to an application rate of 1 lb/acre so that the results are comparable to the GLEAMS-Driver modeling.

FIRST (*F*QPA *I*ndex *R*eservoir *S*creening *T*ool) is a Tier I (i.e., screening level) model developed by the EPA for estimating concentrations of pesticides in surface water (U.S. EPA/OPP 2008). As with the GLEAMS-Driver modeling and for the same reasons (Section 3.2.3.4.3), the concentrations estimated by FIRST for two applications are about twice those estimated for a single application. Consequently, only the single application comparisons are discussed.

Based on the central estimates of exposure, the estimated concentrations from FIRST are similar to those for GLEAMS-Driver based on clay soils. The peak central estimate from FIRST is 23

µg/L versus an estimated peak concentration from GLEAMS-Driver of 18.7 µg/L. The longer-term estimate from FIRST is 6.5 µg/L versus an estimated longer-term concentration from GLEAMS-Driver of 6.05 µg/L. The range of concentrations from the GLEAMS-Driver modeling for both clay and loam soils encompass the PRZM/EXAMS simulations for bulb vegetables from U.S. EPA/OPP/EFED (2005). The ranges from the GLEAMS-Driver modeling (e.g., 1.6-172 µg/L per lb/acre for peak concentrations in clay soils), however, are much greater than those from the FIRST modeling (i.e., 17-43.4 µg/L per lb/acre). Broader ranges from the GLEAMS-Driver modeling relative to both FIRST and PRZM/EXAMS modeling are commonly noted in Forest Service risk assessments and appear to reflect the broader range of input values used in the GLEAMS-Driver modeling, the number and diversity of locations and soil types used in the GLEAMS-Driver modeling, and the large number of simulations conducted in the GLEAMS-Driver modeling relative to the PRZM/EXAMS modeling.

PRZM-GW (Pesticide Root Zone Model for Ground Water) is a Tier 1 model developed by the EPA in conjunction with Canada's Pesticide Management Regulatory Authority to estimate concentrations of pesticides in groundwater. As summarized in Table 13, PRZM-GW estimated concentrations of spinosad in groundwater are substantially below those estimated by GLEAMS-Driver, FIRST, or PRZM/EXAMS. Concentrations of spinosad in groundwater are not specifically used in Forest Service risk assessments; hence, the results from PRZM-GW are noted only for the sake of completeness in terms of covering models commonly used by EPA.

3.2.3.4.5. Monitoring Data

No monitoring data for spinosad are included in compendia published by the U.S. Geological Survey's National Water-Quality Assessment Program (USGS/NAWQA) covering periods from 1992-2001 (Gilliom et al. 2007) or the more recent update covering periods from 1992-2008 (Ryberg et al. 2011). In the conduct of the current Forest Service risk assessment, the California database (http://www.cdpr.ca.gov/docs/emon/surfwtr/surfcont.htm) was searched (April 5, 2016) and no monitoring data were identified. Monitoring studies are not discussed in the EPA or APHIS risk assessments on spinosad (Table 1, Section 1.1).

The one available monitoring study from the California Department of Pesticide Regulation (Fan et al. 2008) is associated with the application of spinosad for the eradication of the Mexican fruit fly in San Diego County during 2003. The application involved a 23 square mile area treated at a rate of 3.26 µg/ft^2 or about 0.0003 lb a.i./acre [3.26 µg/ft^2 x 43560 ft^2/acre = 142,005.6 µg/acre ≈ 0.000142 kg/acre; 0.000142 kg/acre x 2.2046 lb/kg ≈ 0.0003 lb/acre]. Spinosad was not detected in surface water or rain runoff, which is to be expected, given the low application rate. As summarized in Table 13, the direct spray of a small pond at an application rate of 1 lb a.i./acre could result in a concentration of 112 µg/L. At an application rate of 0.0003 lb/acre, the expected concentration would be about 0.03 µg/L [112 µg/L x 0.0003 lb/acre ≈ 0.0336 µg/L]. This concentration is somewhat below the detection limit of 0.05 µg/L for spinosad in water noted by Fan et al. (2008, p. 18).

In terms of evaluating the surface water modeling efforts discussed in the previous sections, the most useful monitoring studies are those that associate monitored concentrations of a pesticide in water with defined applications of the pesticide—e.g., applications at a defined application rate to a well characterized field. When available, such studies can provide a strong indication of the plausibility of modeled concentrations of a pesticide in surface water. In the absence of

monitoring data, the modelled estimates discussed in Sections 3.2.3.4.3 and 3.2.3.4.4 cannot be further evaluated.

3.2.3.4.6. Concentrations in Water Used for Risk Assessment

The modeled surface water concentrations of spinosad used in the current risk assessment are summarized in Table 14. The concentrations are specified as water contamination rates (WCRs)—i.e., the concentrations in water expected at a normalized application rate of 1 lb a.i./acre, converted to units of ppm or mg/L per lb a.i./acre. In Table 13, the summary of all of the modeling efforts, units of exposure are expressed as ppb or µg/L, as a matter of convenience. In Table 14, however, ppb is converted to mg/L (ppm) because mg/L is the unit of measure used in the EXCEL workbooks for contaminated water exposure scenarios in both the human health and ecological risk assessments. The water contamination rates are entered in Worksheet B04Rt in the attachments to this risk assessment. The values in Worksheet B04Rt are linked to the appropriate scenario-specific worksheets in the EXCEL workbooks and are adjusted to the application rate entered in Worksheet A01—i.e., 0.226 lb a.i./acre in the workbooks released with this risk assessment. In the worksheet associated with contaminated surface water, the application rate is multiplied by the water contamination rates to estimate the expected concentrations of spinosad in surface water.

As discussed previously and summarized in Table 13, the Gleams-Driver simulations of the small pond provide the highest estimates of spinosad concentrations in surface water and the central estimates from GLEAMS-Driver are reasonably consistent with the central estimates from the Tier I modeling using FIRST. As detailed in Section 3.2.3.4.3, the GLEAMS-Driver simulations encompass a much broader range of soils and locations with a concomitant increase in the range of modelled values. Consequently, the Gleams-Driver simulations serve as the primary basis for the water concentrations of spinosad used in the current risk assessment.

As noted in 3.2.3.4.5, monitoring data on concentrations of spinosad in surface water are not available to assess the plausibility of the modeling. While the Gleams-Driver estimates are reasonably consistent with U.S. EPA/OPP modeling (Section 3.2.3.4.4), the lack of appropriate monitoring data adds uncertainty to this risk assessment.

As with all uses of GLEAMS-Driver in Forest Service risk assessments, the estimated concentrations of spinosad in water cover a substantial range. For example, the estimated peak concentrations following a single application range from 0.00008 to 0.17 mg/L per lb a.i. applied. This range spans a factor of over 2000 [0.17 ÷ 0.00008 = 2125]. This variability is typical of composite summaries of GLEAMS-Driver simulations in Forest Service risk assessments and reflects the wide range of conditions used in the GLEAMS-Driver modeling (Section 3.2.3.4.3). In region-specific or site-specific assessments, considerations should be given to the more detailed summaries of the modeling simulations in Appendix 8 (one application) or Appendix 9 (two applications) or to conducting site-specific assessments to reflect local conditions (see SERA 2011b, Section 3.3.4).

3.2.3.5. Oral Exposure from Contaminated Fish

Many chemicals may be concentrated or partitioned from water into the tissues of aquatic animals or plants. This process is referred to as bioconcentration. Generally, bioconcentration is measured as the ratio of the concentration in the organism to the concentration in the water. For

example, if the concentration in the organism is 5 mg/kg and the concentration in the water is 1 mg/L, the bioconcentration factor (BCF) is 5 L/kg [5 mg/kg ÷ 1 mg/L]. As with most absorption processes, bioconcentration depends initially on the duration of exposure but eventually reaches steady state.

Three sets of exposure scenarios are presented: one set for acute exposures following an accidental spill (Worksheets D08a and D08b), one set for acute exposures based on expected peak concentrations of spinosad in water (Worksheets D09c and D09d), and another set for chronic exposures based on estimates of longer-term concentrations in water (Worksheets D09a and D09b). The two worksheets for each set of scenarios are included to account for different consumption rates of caught fish among the general population and subsistence populations. Details of these exposure scenarios are provided in Section 3.2.3.5 of SERA (2014a).

The scenarios associated with consumption of contaminated fish are based on the same concentrations of spinosad in water used for the accidental spill scenario (Section 3.2.3.4.1.) and the surface water exposure estimates (Section 3.2.3.4.6).

Generally, bioconcentration factors for the edible portion of fish (i.e., muscle) are used in the human health risk assessment under the assumption that humans will not generally consume offal. As summarized in Table 2, BCFs are available for spinosyn A (MRID 43557601), spinosyn D (MRID 44537734), and total residues of spinosyns A, D, and metabolites (U.S. EPA/OPP/EFED 2009a, p. 8). Consistent with the total residue approach taken for surface water modeling, the current risk assessment uses the BCF for total residues—i.e., bioconcentration factors of 16 to 47 for edible tissue. Given the relationship between exposure time and bioconcentration, the lower bound of 16 is used for acute exposures and the upper bound of 47 is used for longer-term exposures. As noted in Section 4.2.2.5, the BCFs for whole fish are used in the exposure assessments for mammalian and avian wildlife—i.e., a BCF of 84 for acute exposures and a BCF of 115 for longer-term exposures.

3.2.3.6. Dermal Exposure from Swimming in Contaminated Water

Some geographical sites maintained by the Forest Service or Forest Service cooperators include surface water in which members of the general public might swim. The extent to which this might apply to areas treated with spinosad is unclear.

To assess the potential risks associated with swimming in contaminated water, an exposure assessment is developed for a young woman swimming in surface water for 1 hour (Worksheet D10). Conceptually and computationally, this exposure scenario is virtually identical to the contaminated gloves scenario used for workers (Section 3.2.2.2)—i.e., a portion of the body is immersed in an aqueous solution of the compound at a fixed concentration for a fixed period of time.

As in the corresponding worker exposure scenario, the 1-hour period of exposure is intended as a unit exposure estimate. In other words, both the absorbed dose and consequently the risk will increase linearly with the duration of exposure, as indicated in Worksheet D10. Thus, a 2-hour exposure would lead to an HQ that is twice as high as that associated with an exposure period of 1 hour. In cases in which this or other similar exposures approach a level of concern, further

consideration is given to the duration of exposure in the risk characterization (Section 3.4). For spinosad, however, the HQs for this scenario are far below the level of concern.

The scenarios for exposures associated with swimming in contaminated water are based on the peak water concentrations of spinosad used to estimate acute exposure to drinking water (Section 3.2.3.4.6).

3.2.3.7. Oral Exposure from Contaminated Vegetation

Although none of the Forest Service applications of spinosad will involve crop treatment, they may be conducted on some Forest Service lands by individuals or organizations with permission from the Forest Service to use the lands for crop cultivation. All such agricultural applications are subject to U.S. EPA/OPP regulatory constraints (e.g., tolerance limits), and exposures associated with agricultural applications are not explicitly considered in Forest Service risk assessments.

For pesticides that may be applied to vegetation, Forest Service risk assessments include standard exposure scenarios for the acute and longer-term 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, detailed in Worksheets D03a (fruit) and D03b (vegetation) for acute exposure and Worksheets D04a (fruit) and D04b (vegetation) for chronic exposure. The key inputs for these scenarios are the initial residues on the vegetation and the amount of fruit or vegetation consumed for both acute and chronic scenarios. For chronic scenarios, additional key inputs are the half-lives of the pesticide on the fruit or vegetation as well as the period used to estimate the average concentration of the pesticide on vegetation.

In most Forest Service risk assessments, the initial concentration of the pesticide on fruit and vegetation is estimated using the empirical relationships between application rate and concentration on different types of vegetation (Fletcher et al. 1994). These residue rates are summarized in Table 15. The rates provided by Fletcher et al. (1994) are based on a reanalysis of data originally compiled by Hoerger and Kenaga (1972) and represent estimates of pesticide concentration in different types of vegetation (mg chemical/kg vegetation) at a normalized application rate of 1 lb a.i./acre. Although the EPA human health risk assessments do not consider exposure scenarios involving direct spray, the residue rates recommended by Fletcher et al. (1994) are used by U.S. EPA/OPP/EFED in their T-REX exposure model for terrestrial organisms (http://www.epa.gov/oppefed1/models/terrestrial/trex/t_rex_user_guide.htm).

Table 15 also summarizes two residue studies on spinosad, one study on cauliflower (Mandel et al. 2009) and the other study on cowpea pods (Vijayasree et al. 2014). As detailed in Table 15, both studies yield estimated residue rates of about 15 mg a.i./kg food) per lb a.i./acre. The study by Vijayasree et al. (2014) on cowpea pods is consistent with the upper bound estimate of 15 mg a.i./kg food) per lb a.i./acre for fruits, pods, seeds, and large insects from Fletcher et al. (1994). Cauliflower is essentially a variety of cabbage, which would typically be classified as a broadleaf. The residue rates for cauliflower of about 14 to 16 a.i./kg cauliflower from Mandel et al. (2009) are near the lower bound for broadleaf plants from Fletcher et al. (1994). While based on only two studies, the reasonable concordance of pesticide-specific residues rates with the rates from Fletcher et al. (1997) is a common pattern noted in Forest Service risk assessments. This

concordance is reasonable because residue rates should largely depend on application rate and leaf area index. It is reasonable to expect that residue rates will not vary substantially for most pesticides, with the possible exception of highly volatile pesticides (which do not include spinosad). Consequently and as in most Forest Service risk assessments, the residues rates from Fletcher et al. (1997) summarized in Table 15 are used to estimate the initial residues of spinosad on vegetation.

The half-lives on vegetation used in chronic exposure scenarios are based on the same rates used in GLEAMS-Driver modeling (Table 12)—i.e., 6 days with a range of 1.5 to 35 days. The central estimate is approximated from Sharma et al. (2008, high application rate) and lower bound values from Tomkins et al. (1991). As summarized in Table 2, several foliar half-lives are reported in the open literature with values ranging from about 1.5 days (Mandal et al. 2009; Sharma et al. 2008; Singh and Battu 2012) to about 16 days (Tomlin 2004; Tomkins et al. 1991). The lower bound half-life of 1.5 days is taken from Vijayasree et al. (2014, cowpea). While a half-life of 16 days could be used based on the values for spinosad reported in the literature, the upper bound value of 35 days is taken in deference to U.S. EPA/OPP/EFED (2011a), which uses a default half-life of 35 days in an ecological risk assessment on spinosad.

Based on these half-lives on vegetation and fruit, the longer-term concentrations of the pesticide in various commodities are detailed in Worksheets B05a (fruit), B05b (broadleaf vegetation), B05c (short grass), and B05d (long grass). Only the worksheets for fruit and broadleaf vegetation are used in the human health risk assessment. All four worksheets are used in the ecological risk assessment (Section 4.2). In all cases, a maximum 90-day time-weighted average concentration is calculated for longer-term exposures. In the context of the human health risk assessment, the use of the 90-day rather than a 365-day time-weighted average is intended to reflect the harvesting of a 1-year supply of fruit and/or vegetation during a single season (i.e., about 90 days) under the assumption that degradation will not occur once the commodity is harvested—e.g., the commodities are placed in cold storage, which essentially stops the degradation of the pesticide.

As summarized in Worksheet E03 of Attachment 1 (single application), the estimated acute exposures are 0.00265 (0.00121 – 0.042) mg/kg bw for the consumption of contaminated fruit and 0.0365 (0.00253-0.34) mg/kg bw/day for the consumption of contaminated vegetation. The estimated longer-term exposures are 0.000254 (0.0000291-0.0196) mg/kg bw/day for contaminated fruit and 0.00351 (0.000061-0.142) mg/kg bw/day for contaminated vegetation. As summarized in Worksheet E03 of Attachment 2 (two applications), the estimated doses for contaminated fruit and vegetation are somewhat less than a factor of 2 higher than the doses associated with a single application. This is to be expected given the short interval between applications (i.e., 6 days).

The U.S. EPA/OPP approach to dietary exposure is different from the approach used in Forest Service risk assessments. While Forest Service risk assessments consider the consumption of fruit and vegetation directly sprayed with a pesticide, the EPA exposure assessments are based on dietary surveys (i.e., the amounts of different commodities consumed by individuals) and tolerance limits on those commodities—i.e., the concentration of vegetation used in the exposure assessment assumes that the tolerances set by EPA are not exceeded. In EPA's most recent

human health risk assessment document (U.S. EPA/OPP/HED 2011a, Table A.7.1, p. 60), estimates of total chronic dietary exposures for humans of different age groups range from about 0.0018 to 0.0059 mg/kg bw/day. These estimates are similar to the central estimates of chronic exposures derived in the current risk assessment for the consumption of contaminated vegetation. This similarity, however, is coincidental. More significantly in terms of the interpretation of potential risk, the upper bound estimates of dietary exposure given the current risk assessment are substantially higher than those presented by EPA. This is a common pattern in Forest Service risk assessments and reflects the different methods and scenarios used in Forest Service risk assessments, relative to the methods used by EPA.

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview

Table 16 provides an overview of the dose-response assessment used in this risk assessment. Following standard practices in Forest Service risk assessments, RfDs are adopted from the values proposed by U.S. EPA.

The U.S. EPA, WHO and other European organizations determined that no acute RfD or comparable value is required for single-day exposures. The EPA, however, uses a subchronic oral NOAEL of 4.9 mg/kg bw with a recommended Margin of Exposure (MOE) of 100 for risk characterization of short-term exposures to spinosad covering periods of 1 to 30 days. This approach is adopted in the current risk assessment using a surrogate acute RfD of 0.049 mg/kg bw/day. This is an admittedly conservative and perhaps overly protective approach that is considered further in the risk characterization.

The EPA derived two chronic RfDs for spinosad. Initially, the chronic RfD was set at 0.0268 mg/kg bw/day based on a chronic toxicity study in dogs with spinosad. Subsequently, the EPA recommended a chronic RfD of 0.0249 mg/kg bw/day based on a chronic toxicity study in dogs with spinetoram. The European Commission recommends a chronic ADI (essentially identical to a chronic RfD) of 0.024 mg/kg bw/day based on a chronic study in rats with spinosad. Given their similarities, all three of these toxicity values may be viewed as mutually reinforcing, and using any of them would have no impact on the risk characterization for longer-term exposures. Following standard practice in Forest Service risk assessments and in the absence of a compelling reason to do otherwise, the current risk assessment adopts the most recent chronic RfD from EPA—i.e., the chronic RfD of 0.0249 mg/kg bw/day from U.S. EPA/OPP/HED (2007a).

Dose-severity relationships for spinosad are limited by the lack of quantitative data on toxicity in humans and by the limited number of mammalian species on which data are available. Within these constraints, exposures associated with hazard quotients of about 2 might raise concern for covert toxic effects. Based on allometric relationships for chronic LOAELs in mice, rats, and dogs, hazard quotients of 18 might be a more reasonable estimate of exposure levels possibly associated with covert adverse effects. There is no basis for asserting that these exposures, however, would result in frank signs of toxicity. Levels of exposure to spinosad that might result in overt signs of toxicity in humans cannot be estimated with confidence. As discussed further in Section 3.4, this limitation does not have a substantial impact on the current risk assessment in terms of characterizing risks to workers or members of the general public.

3.3.2. Acute RfD

The U.S. EPA/OPP sometimes derives acute RfDs for pesticides. For spinosad, however, the EPA did not derive an acute RfD for the general population. The rationale for not doing so is as follows: *Toxicological effect attributable to a single dose was not identified in the spinosad and spinetoram databases* (U.S. EPA/OPP/HED (2011a, Table A.2.1, p. 35). The recent risk assessment by the European Food Safety Authority reaches essentially the same conclusion: *No ARfD* [acute RfD] *value was deemed necessary for spinosad… due to the low acute toxicity of the active substance* (EFSA 2013, p. 2 and p. 21). The same point is reflected in the FAO/WHO

(2001, p. 53) review of spinosad: *In studies with repeated doses, no acute toxicological alerts were observed that might indicate the need for establishing an acute reference dose*.

As detailed in Section 3.2, several accidental and non-accidental exposure scenarios typically used in Forest Service risk assessments are developed for spinosad. All of these exposure assessments involve exposure for a single day or during a single incident. In the absence of an acute RfD associated with a single day or single incident exposure, the current Forest Service risk assessment uses the approach developed by EPA for short-term incidental exposures (1-30 days). The U.S. EPA/OPP/HED (2009a, Table 3.1, p. 21) assesses such short-term incidental exposures using the NOAEL of 4.9 mg/kg bw/day form a 90-day feeding study in dogs (i.e., MRID 43444102 as summarized in Appendix 1, Table A1-2). In applying this NOAEL to risk characterization, the EPA uses a Margin of Exposure (MOE) of 100, which is based on a factor of 10 for extrapolating from animals to humans multiplied by a factor of 10 considering sensitive subgroups in the human population, which is fundamentally equivalent to a short-term RfD of 0.049 mg/kg bw. This approach is maintained by EPA in their most recent human health risk assessment scoping document (U.S. EPA/OPP/HED 2011a, Table A.2.1). In the absence of an acute RfD, the short-term equivalent RfD of 0.049 mg/kg bw is used to characterize risks associated with acute exposures in the current risk assessment.

The above approach is obviously conservative, and perhaps overly so, because this acute toxicity value is based on a subchronic study but is applied to single-day exposure scenarios. This issue is considered further in the risk characterization (Section 3.4).

3.3.3. Chronic RfD

No chronic RfD for spinosad is available at the EPA's Integrated Risk Information System (IRIS) (https://www.epa.gov/iris). U.S. EPA/OPP derives two chronic RfDs for spinosad. An ADI for spinosad is also derived by EFSA (2013).

Originally, the EPA derived a chronic RfD of 0.0268 mg/kg bw/day based on the chronic study of spinosad in dogs (U.S. EPA/OPP/HED 1997b, p. 13). As summarized in Appendix 1, Table A1-2, this study defines a NOAEL of 2.68 mg/kg bw/day and a LOAEL of 8.36 mg/kg bw/day based on changes in clinical chemistries and tissue pathology (Harada 1995, MRID 43701504).

In a 2007 chronic dietary exposure assessment for both spinosad and spinetoram, the EPA elected to base the chronic RfD for spinosad on a chronic study of spinetoram in dogs. As discussed in U.S. EPA/OPP/HED (2007a, p. 10), this decision is based on the determination that …*spinosad and spinetoram are toxicologically equivalent*. As also summarized in Appendix 1, Table A1-2, the EPA uses a NOAEL of 2.49 mg/kg bw/day from a 1-year feeding study in dogs (MRID 47011901). This study defines a LOAEL of about 5.5 mg/kg bw/day based on tissue pathology. U.S. EPA/OPP/HED (2007a) does not cite the previous RfD or offer a discussion of the rationale for selecting the study on spinetoram over the study on spinosad. While somewhat speculative, the EPA's decision appears to reflect both the determination of the toxicological equivalence of spinosad and spinetoram and the somewhat lower LOAEL in the study on spinetoram (5.5 mg/kg bw/day) relative to the study on spinosad (8.36 mg/kg bw/day). The EPA derives a chronic RfD of 0.0249 mg/kg bw/day using an uncertainty factor of 100 as in the earlier chronic RfD and for the same reasons. This chronic RfD is maintained in the most recent EPA human health risk assessment document (U.S. EPA/OPP/HED 2011a, p. 35).

The European Commission recommends a chronic Acceptable Daily Intake (ADI) of 0.024 mg/kg bw/day based on a chronic study in rats and a "Safety Factor" of 100 (European Commission 2006, Appendix II, p.8). Note that ADIs and RfDs are functionally identical and the term "Safety Factor" is used in the European literature as a functional synonym for the term "Uncertainty Factor" used in most of the U.S. literature. This RfD is maintained in the most recent review of spinosad by the European Food Safety Authority (EFSA 2013, p. 2). The study on which the European ADI is based is not identified in European Commission (2006) or EFSA (2011, 2012, 2013) documents. Based on the review of spinosad by WHO/FAO (2001, p. 58), the chronic study in rats used by the European Commission (2006) appears to be the study by Bond et al. (1995b MRIDs 43701507 and 43710503). As summarized in Appendix 1, Table A1-2, the EPA evaluated this study and determined a NOAEL of 9.5 mg/kg bw/day (i.e., the dose for males in the 200 ppm exposure group). FAO/WHO (2001) classifies 9.5 mg/kg bw as a LOAEL and 2.4 mg/kg bw/day (i.e., the 50 ppm exposure group) as a NOAEL. As detailed in Appendix 1, Table A1-2, the FAO/WHO classification is consistent with the DER for this study from EPA.

The three chronic toxicity values are remarkably similar: 0.0268 mg/kg bw/day (RfD from U.S. EPA/OPP/HED 1997b based on a chronic dog study with spinosad), 0.0249 mg/kg bw/day (RfD from U.S. EPA/OPP/HED 2007a based on a chronic dog study with spinetoram), and 0.024 mg/kg bw/day (ADI from European Commission 2006 based on a chronic study in rats using spinetoram). All three of these toxicity values may be viewed as mutually reinforcing, and the use of any of these toxicity values would have no impact on the risk characterization for longer-term exposures (Section 3.4). Following standard practice in Forest Service risk assessments and in the absence of a compelling reason to do otherwise, the current risk assessment adopts the most recent toxicity value from U.S. EPA—i.e., the chronic RfD of 0.0249 mg/kg bw/day from U.S. EPA/OPP/HED (2007a).

3.3.4. Dose-Severity Relationships

Forest Service risk assessments sometimes consider dose-severity relationships to more fully characterize potential risks in exposure scenarios where the doses exceed the RfD. For spinosad, this consideration is relevant because some of the exposure scenarios for members of the general public lead to estimated doses, particularly at the upper bounds of exposures, which substantially exceed the RfDs (Section 3.4).

As summarized in Table 16, the ratios of the LOAEL to the corresponding NOAEL are about 2 for both the acute RfD [9.73 mg/kg bw/day ÷ 4.9 mg/kg bw/day ≈ 1.9857] and the chronic RfD [5.36 mg/kg bw/day ÷ 2.49 mg/kg bw/day ≈ 2.1526]. While these ratios might not reflect dose-severity responses in human populations, they are the most objective basis for assessing potential concerns for exceedances in the RfDs.

An additional factor to consider in dose-severity considerations is the uncertainty factor of 100 used in the derivation of all of the RfDs. A simple comparison of LOAELs for NOAELs does not consider the impact of uncertainty factors which are intended to be protective—i.e., should generally result in an overestimate of underlying risk. Thus, while hazard quotients of 2 for acute and chronic exposures might be viewed with concern based on the LOAEL to NOAEL ratios, the uncertainty factor of 100 may diminish this concern, if the uncertainty factor is highly protective. In other words, the uncertainty factor is intended to protect sensitive subgroups and

to account for human to animal extrapolation; nonetheless, the uncertainty factor and consequent RfD are not intended as precise adjustments to a human equivalent dose.

For the chronic RfD, the potential impact of conservative uncertainty factors may be explored, if not necessarily refined, based on the species-to-species relationships illustrated in Figure 5. As discussed in Section 3.1.5, the chronic LOAELs for mice, rats, and dogs are well described by a standard allometric function. Based on this relationship, the LOAEL for a 70 kg mammal would be estimated at about 4.5 mg/kg bw/day. This cannot be directly compared to the chronic RfD because the RfD includes factors of 10 for both animal-to-human extrapolation as well as sensitive individuals within the population. The allometric relationship may account for the factor of 10 used for animal-to-human extrapolation but not the factor for sensitive individuals. Apply the factor of 10 for sensitive individuals, the estimated LOAEL for humans would be 0.45 mg/kg bw/day. As discussed in Section 3.3.3, the chronic RfD used in the current risk assessment is 0.0249 mg/kg bw/day. Thus, the allometric relationship for the chronic toxicity of spinosad suggests that a hazard quotient of about 18 [0.45 mg/kg bw/day ÷ 0.0249 mg/kg bw/day ≈ 18.0722] would be viewed as an unacceptable exposure. Based on effects observed at the LOAEL in the chronic study on dogs, adverse effects could include organ pathology; however, overt toxic effects might not be observed.

Levels of exposure that might be associated with frank signs of toxicity cannot be clearly determined based on the limited human data on spinosad (i.e., Su et al. 2011 as discussed in Section 3.1.4.2) and low acute toxicity of spinosad by all routes of administration (Sections 3.1.4.1, 3.1.12, and 3.1.13). For the current risk assessment, these limitations in assessing dose-severity relationships at exposures exceeding a hazard quotient of about 18 (discussed above) are not a practical concern. As discussed further in Section 3.4, the highest hazard quotient in the risk characterization is 15.

3.4. RISK CHARACTERIZATION

3.4.1. Overview

The risk characterizations for workers (Worksheet E02) and members of the general public (Worksheet E04) are summarized in the attachments to this risk assessment—i.e., Attachment 1 for a single application and Attachment 2 for two applications with a 6-day application interval. All risk characterizations are based on an application rate of 0.225 lb a.i./acre.

Consistent with the EPA occupational risk assessments, none of the estimates for general exposures of workers developed in the current risk assessment results in HQs that exceed the level of concern (HQ=1) even at the upper bounds. Similarly, none of the accidental exposure scenarios for workers approach a level of concern. A residual concern for workers involves the potential for eye irritation. While the studies reviewed by EPA do not suggest that spinosad is likely to be an eye irritant and none of the product labels requires eye protection, the MSDS/SDS for some formulations suggest the potential for moderate to serious eye irritation, and all of the MSDS/SDS recommend the use of protective eyewear. Prudence suggests that this cautionary language on the MSDS/SDS should be considered in any application of these formulations.

The only non-accidental exposure scenarios for members of the general public that exceed the level of concern involve the consumption of contaminated vegetation (following a single application or two applications) and the consumption of contaminated fruit (following two applications). The HQs that exceed the level of concern range from 1.1 to 12. Based on dose-severity relationships, the HQ of 1.1 (the central estimate of exposure for the consumption of contaminated vegetation following two applications) does not raise substantial concern. While the upper bound HQs associated with contaminated vegetation or fruit (i.e., HQs from 1.6-12) would probably not be associated with frank signs of toxicity, the levels of exposure are in excess of exposures that would be considered acceptable. If spinosad is sprayed on vegetation that might be consumed by humans, measures to mitigate exposures to members of the general public should be considered.

HQs associated with accidental exposure scenarios for members of the general public do not exceed the level of concern for direct spray; nevertheless, some HQs for the accidental spill scenarios do exceed the level of concern with a maximum HQ of 15 (i.e., the consumption of contaminated fish by subsistence populations). While there is no direct evidence that these scenarios would result in observable signs of toxicity, these HQs justify measures to reduce/mitigate exposures in members of the general public.

Spinosad shares a common mechanism of action with spinetoram, and the two insecticides are considered to be toxicologically equivalent. If spinosad and spinetoram are used concurrently in the same location, the cumulative effects of both insecticides should be considered quantitatively. Spinosad may enhance the toxicity of other compounds, possibly via an inhibition of P-glycoprotein or competition with cytochrome P450. P-glycoprotein and cytochrome P450 play significant roles in the metabolism and/or elimination of a wide variety of compounds, both naturally occurring and synthetic. Thus, spinosad could interact toxicologically with other compounds. The occurrence and nature of any interactions would depend on the levels of exposure and the specific mechanism(s) for any interactions between

spinosad and the other compounds. Further generalizations are not warranted by the available information.

3.4.2. Workers

The highest HQs for workers are 0.2, the upper bound HQs for workers involved in ground broadcast and aerial applications. These HQs are below the level of concern (HQ=1) by a factor of 5. Note that the accidental HQs are lower than the HQs for general exposures. As discussed in Section 3.2.2.2, all of the accidental exposure scenarios for workers involve dermal exposures. Given the poor dermal absorption of spinosad (Section 3.1.3.2) and low dermal toxicity of spinosad (Section 3.1.12), dermal exposures are not expected to pose a hazard.

The benign risk characterization for workers is qualitatively similar to the risk characterizations for workers given in EPA risk assessments. In the most recent completed risk assessment for workers, the EPA maintains that . . . *risks* [to workers] *are not of concern* (U.S. EPA/OPP/HED 2009a, p. 38). This language is also reflected in the EPA's scoping document for the registration review of spinosad (U.S. EPA/OPP/HED 2011a, p. 22).

The only reservation in the risk characterization involves the potential for eye irritation. As discussed in Section 3.1.11.3, U.S. EPA/OPP/HED (1997b, 2009a, 2010b, 201 la) categorizes both technical grade spinosad and an unspecified 44% a.i. formulation of spinosad as Category IV (i.e., the least severe category) for eye irritation. Nonetheless, as summarized in Table 4, the Safety Data Sheet for the Entrust [80% a.i., WP] formulation is atypical in indicating that the formulation ...*Causes serious eye irritation.* In addition, the SDS for Entrust as well as the SDS for Conserve SC (11.6% a.i.) and SpinTor 2SC (22.8% a.i.) indicate that the formulations ...*May cause pain disproportionate to the level of irritation to eye tissues.* While not required on the product labels, the MSDS/SDS for the representative formulations considered in the current risk assessment (Table 4) recommend the use of protective eyewear. Prudence suggests that this cautionary language on the MSDS/SDS should be considered in any application of these formulations.

3.4.3. General Public

The HQs associated with the consumption of contaminated vegetation and fruit following applications of spinosad are the only HQs that exceed the level of concern (HQ=1). This is a common pattern in risk assessments in which the pesticide is applied to vegetation that might be consumed by humans. As discussed in Section 3.2.3.7, the estimated doses for two applications at an application interval of 6 days (Attachment 2) are somewhat less than twice that of residues following a single application (Attachment 1). Because HQs are linearly related to dose, the HQs for two applications are somewhat less than a factor of two below the HQs for a single application.

At the central estimates, none of the HQs following a single application exceeds the level of concern. For two applications, the HQ for the consumption of contaminated vegetation (HQ=1.1) modestly exceeds the level of concern. At the upper bounds, the acute and chronic HQs for the consumption of contaminated vegetation following a single application are identical (HQ=6). Following two applications, the upper bound HQ for the consumption of contaminated vegetation for acute exposure (HQ=12) is twice that for a single application. For longer-term

exposures, the HQ is somewhat less than twice that following a single application (HQ=11). For clarity, it is noted that all HQs equal to or greater than 2 are rounded to the nearest significant digit as a convention (SERA 2011b, p. 17). The upper bound of the underlying chronic HQ for the consumption of contaminated vegetation following a single application without rounding is about 5.693 and the upper bound of the corresponding HQ without rounding following two applications is about 11.24. Thus, at least in terms of the underlying unrounded values, the HQ for two applications is about twice the HQ for a single application [$11.24 \div 5.693 \approx 1.974$]. The only other HQ that exceeds the level of concern is the upper bound HQ for the consumption of contaminated fruit following two applications (HQ=1.6).

As discussed in Section 3.3.4, HQs above 2 would be associated with LOAELs in experimental mammals based on the ratio of the LOAEL to the NOAEL. In other words, HQs in excess of 2 could raise concern for covert adverse effects. Based on allometric relationships for chronic toxicity, HQs of up to 18 could be associated with covert adverse effects but not with signs of frank toxicity. Levels of exposure that might be associated with overt adverse effects cannot be identified. Based on these relationships, the modest exceedance (HQ=1.1) based on the central estimate of exposure for the consumption of contaminated vegetation following two applications does not raise substantial concern. While the upper bound HQs in the range of 1.6 to 12 associated with contaminated vegetation or fruit would probably not be associated with frank signs of toxicity, the levels of exposure are in excess of exposures that would be considered acceptable. If spinosad is sprayed on vegetation that might be consumed by humans, measures to mitigate exposures to members of the general public would be prudent.

The accidental exposures associated with direct spray are below the level of concern (i.e., a maximum HQ of 0.2). Accidental spills, however, lead to HQs of up to 15 (i.e., the consumption of contaminated fish by subsistence populations). As with the non-accidental exposures, there is no basis for asserting that accidental spills would lead to overt toxic effects in members of the general public. Nonetheless, these HQs justify measures to reduce/mitigate exposures.

3.4.4. Sensitive Subgroups

For 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 spinosad. Under the Food Quality Protection Act (FQPA), the EPA is required to consider populations that might be at increased risk to pesticide exposures including considerations of reproductive effects, neurologic effects, and effects on immune function. Each of these effects is considered in Section 3.1. Consistent with the current risk assessment, the EPA determined that these endpoints do not justify quantitative changes in the dose-response assessment (U.S. EPA/OPP/HED 2011a, p. 6).

Given the available information on spinosad, subgroups in the human population that might be atypically sensitive to spinosad have not been identified.

3.4.5. Connected Actions

The Council on Environmental Quality (CEQ), which provides the framework for implementing NEPA, defines connected actions as actions which occur in close association with the action of concern; in this case, the use of a pesticide (40 CFR 1508.25, https://ceq.doe.gov/nepa/regs/ceq/1508.htm).

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 spinosad, "connected actions" include actions or the use of other chemicals which are necessary and occur in close association with use of spinosad.

Spinosad formulations contain inert components, and the metabolism of spinosad may involve the formation of a number of different compounds. Thus, spinosad applications will entail (automatically trigger) exposures to inerts as well as metabolites. As discussed in detail in Sections 3.1.14 (Inerts and Adjuvants), the disclosed inerts in spinosad formulations do not appear to present hazards that require quantitative consideration. As discussed in Section 3.1.15 (Impurities and Metabolites) and implemented in the exposure assessments (Sections 3.2 and 4.2), the metabolites of spinosad are explicitly considered using the total residue approach similar to that employed in U.S. EPA/OPP/HED (2011a, p. 9).

3.4.6. Cumulative Effects

Cumulative effects may involve either repeated exposures to an individual agent or simultaneous exposures to the agent of concern (in this case spinosad) and other agents that may cause the same effect or effects by the same or a similar mode of action.

The U.S. EPA/OPP makes the following assessment of cumulative risk for both spinosad and spinetoram:

> *Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as to spinetoram/spinosad and any other substance and spinetoram/spinosad do not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance action, therefore, EPA has not assumed that spinetoram/ spinosad does not have a common mechanism of toxicity with other substances.*
>
> U.S. EPA/OPP (2009a, pp. 32)

The human health risk assessment scoping document contains similar language and indicates that the EPA will review any new information relating to potential cumulative risks with other pesticides (U.S. EPA/OPP/HED 2011a).

Explicit in the above determination, as discussed in Section 3.1.5, the EPA has determined that spinosad and spinetoram have a common mechanism of action and are toxicologically equivalent. Thus, if spinosad and spinetoram are used concurrently in the same location, the cumulative effects of both spinosad and spinetoram should be considered quantitatively.

As discussed in Section 3.1.16 (Toxicological Interactions), spinosad may enhance the toxicity of other compounds (e.g., ivermectin and milbemycin oxime), possibly via an inhibition of P-glycoprotein or competition with cytochrome P450. P-glycoprotein and cytochrome P450 play significant roles in the metabolism and/or elimination of a wide-variety of compounds, both

naturally occurring and synthetic. The occurrence and nature of any interactions will depend on the levels of exposure as well as the specific mechanism(s) for any interactions between spinosad and the other compounds. Further generalizations are not warranted by the available information.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview

Spinosad is an effective insecticide used to control numerous insects. Spinosad is much more toxic to insects than to vertebrates with LD_{50} values in insects ranging from about 0.025 to 65 mg/kg bw versus LD_{50} values in mammals and birds greater than 1000 mg/kg bw. There is substantial variability in the toxicity of spinosad to different groups of insects. The most sensitive orders of insects appear to be nontarget Hymenoptera (particularly bees and parasitic wasps) as well as target species of Diptera, and Lepidoptera. As might be expected for an insecticide typically applied to vegetation, terrestrial macrophytes are not adversely affected by spinosad. Based on limited data on earthworms, the toxicity of spinosad to terrestrial invertebrates appears to be limited to arthropods.

As with terrestrial organisms, sensitive species of aquatic arthropods are more vulnerable than sensitive species of aquatic vertebrates (i.e., fish) to spinosad exposure. The differences in sensitivity among tolerant species of aquatic arthropods and tolerant species of fish are minor. The differences in sensitivity are more pronounced, however, among sensitive species of fish and sensitive species of aquatic invertebrates. Most but not all species of algae are relatively tolerant to spinosad exposures. One exception is the freshwater diatom, *Navicula pelliculosa*, which is more sensitive than sensitive species of fish to spinosad.

4.1.2. Terrestrial Organisms

4.1.2.1. Mammals

The toxicity studies used to assess the potential hazards of spinosad to humans (Section 3.1 and Appendix 1) are applicable to the risk assessment for mammalian wildlife. While the human health risk assessment typically focusses on the most sensitive species, the ecological risk assessment attempts to identify subgroups of mammals that may display greater or lesser sensitivity to a particular pesticide. These differences may be based on allometric scaling (e.g., Sample and Arenal 1999) or differences in physiology. As discussed in Section 3.1.5 and illustrated in Figure 5, dogs appear to be more sensitive than rats and mice based on chronic LOAELs, and the relationship fits a standard allometric function indicating that larger mammals may be more sensitive than smaller mammals. While dogs appear to be more sensitive than rats and mice based on subchronic LOAELS, the data are scattered and do not fit an allometric model. As summarized in Appendix 1, Table A1-1, acute LD_{50} values are available only on mice and rats; furthermore, most of the LD_{50} values are indefinite—i.e., expressed as greater than a given dose. Definitive LD_{50} values in male mice (6100 mg/kg bw from FAO/WHO 2001) and male rats (3738 mg/kg bw from MRID 43414515) support the supposition that larger mammals may be more sensitive than smaller mammals; however, the two data points do not permit an assessment of the statistical significance of this relationship—i.e., application of the allometric model (2 parameters) to two data points leads to zero degrees of freedom. Developmental studies were conducted with rabbits and rats (Appendix 1, Table A1-3); however, both studies fail to define adverse effect levels. Thus, potential differences in sensitivities between rabbits and rats cannot be assessed.

While the available data are not compelling, dogs and other canids are identified as a subgroup of mammals that may be more sensitive than rodents to spinosad exposure. This issue is addressed further in the dose-response assessment for mammals. Given the limitations in the allometric relationships in the chronic LOAEL studies and the lack of an allometric relationship in the subchronic LOAEL studies, separate toxicity values are not derived for small and large non-canid mammals.

4.1.2.2. Birds

As summarized in Appendix 2, a standard set of toxicity studies—i.e., acute gavage studies (Appendix 2, Table A2-1), acute dietary studies (Appendix 2, Table A2-2), and reproduction studies (Appendix 2, Table A2-3) were submitted to the U.S. EPA/OPP in support of the registration of spinosad.

The acute gavage studies in birds typically involve the administration of single doses with a 14-day observation period (U.S. EPA/OCSPP 2012a). The gavage studies in mallards (Murray 1992, MRID 43414528) and quail (Murray et al. 1992b, MRID 43414529) are somewhat atypical in that three doses were administered over a 6-hour period. As noted in the DERs for these studies, the full studies submitted to EPA do not provide a rationale for the multiple doses. As detailed in Appendix 2, Table A2-1, the total doses administered to the birds were 0, 200, 500, 1000, or 2000 mg/kg bw, none of which caused mortality—i.e., the LD_{50} could be specified as >2000 mg/kg bw, which is how the LD_{50} values are specified for mallards and quail in the review by the European Commission (2006, p. 24). This characterization of the LD_{50} would result in a classification of spinosad as Practically Nontoxic (e.g., SERA 2014a, Table 16). Because the doses were spaced over a 6-hour period, however, the EPA designates the maximum dose at 1333 mg/kg bw (2/3 x 2000) and classifies spinosad as *Slightly Toxic* (U.S. EPA/OPP/EFED 2011a, p. 35). While mortality was not observed in either study, quail evidenced signs of toxicity at all but the lowest dose (i.e., NOAEL = 200 mg/kg bw). No signs of toxicity were observed in mallards at doses up to 2000 mg/kg bw. Thus, quail appear to be more sensitive than mallards to spinosad. As discussed in Section 3.1.6, spinosad does not appear to be neurotoxic in mammals. While the study in quail does note ataxia in quail at doses of 500 mg/kg bw and above, it is not clear if the ataxia can be regarded as a direct neurotoxic effect. Nonetheless, in the absence of other signs of toxicity, the occurrence of ataxia is suggestive of neurotoxicity in quail.

The acute dietary studies in mallards (Murray and Woolwine 1992, MRID 43414530) and quail (Murray et al. 1992a, MRID 43414531) are similar to the acute gavage studies in that the reported LC_{50} values are indefinite, specifically >5156 ppm for both mallards and quail. Based on the LC_{50} values, the EPA classifies spinosad as *Practically Nontoxic* to birds in terms of acute dietary exposures (U.S. EPA/OPP/EFED 2011a, p. 36). Also as with the acute gavage studies, quail appear to be more sensitive than mallards to spinosad. As detailed in Appendix 2, Table A2-2, there was no mortality or signs of toxicity in mallards exposed to dietary concentrations of up to 5156 ppm. In quail, signs of toxicity included decreased body weight at concentrations of 1335 ppm and above, loose feces at concentrations of 5253 ppm, and mortality (1/10) at concentrations of 2601 and 5252 ppm. The NOAEL for quail was 656 ppm. As detailed in Appendix 2, Table A2-2, the NOAEL of 656 ppm corresponds to a dose of about 200 mg/kg bw/day based on approximate food consumption rates from similar studies on other pesticides

for which food consumption rates are available [656 mg/kg food x 0.3 kg food/kg bw = 196.8 mg/kg bw]. This estimated dietary NOAEL of 200 mg/kg bw in quail is identical to the NOAEL from the gavage study in quail (as discussed in the previous paragraph).

Unlike the acute toxicity studies, there are no remarkable sensitivity differences among quail and mallards demonstrated in the available reproduction studies (Appendix 2, Table A2-3). In both species, adverse reproductive effects were noted at 1100 ppm. The reproductive effects were severe and characterized by decreases in live embryos and offspring survival in both species. Also in both species, no adverse effects on adults, offspring, or reproductive parameters were noted at 550 ppm. The DERs for the study in quail (Beavers et al. 1994a, MRID 43414533) and the study in mallards (Beavers et al. 1994b, MRID 43414532) do not provide sufficient information to estimate doses in units of mg/kg bw/day. For both mallards and quail, dietary concentrations (mg/kg diet) are converted to mg/kg bw/day doses using a food consumption factor of 0.07 kg food/kg bw based on reproduction studies in quail and mallards (SERA 2007b). Based on this food consumption factor, the dietary NOAEC of 550 mg a.i./kg diet corresponds to a dose of about 38.5 mg/kg bw/day [550 mg/kg food x 0.07 kg food/kg bw = 38.5 mg/kg bw] and the LOAEC corresponds to a dose of about 77 mg/kg bw/day [1100 mg/kg food x 0.07 kg food/kg bw = 77 mg/kg bw]. As discussed in Section 3.1.9.2, the LOAEL in birds is similar to the LOAEL of 100 mg/kg bw/day in rats (Breslin et al. 1994, MRIDs 43701506) which is based on comparable endpoints—i.e., decreases in litter size and offspring survival. The NOAEL in mammals (10 mg/kg bw/day) is lower than the estimated LOAEL in birds (38.5 mg/kg bw/day); nevertheless, this difference may be an artifact of the dose spacing in the studies rather than a true difference in sensitivity between mammals and birds.

The avian toxicity studies in the open literature on spinosad do not substantially expand the information directly useful in the hazard identification for birds. In an abstract of a residue feeding study in hens, Magnussen et al. (1996) note that spinosad accumulates primarily in the liver and fat with metabolites reflecting N-demethylation or O-demethylation. This general pattern is similar to that in mammals (Section 3.1.3.1). Spinosad is used in poultry production for the control of the poultry red mite, *Dermanyssus gallinae*. In this use, spinosad solutions in the range of 2000 to 4000 mg a.i./L are sprayed in nesting facilities. Studies documenting the efficacy of this use (e.g., George et al. 2010; Leibisch et al. 2011) do not indicate adverse effects on hens or egg production. Given the nature of the exposures, estimates of doses, in units of mg/kg bw, to the chickens in treated facilities cannot be made. Uggini et al. (2012) examined the effect of spinosad solutions on chicken eggs via direct injection. While adverse effects were not noted at spinosad concentrations of 100 µg/egg, deformities (skull, sternum, and ribcage) were noted at doses of 500 and 750 µg/egg. Again, these estimates of exposure are not directly comparable to the data from the reproduction studies in birds (discussed above).

4.1.2.3. Reptiles and Amphibians (Terrestrial-Phase)

There are no data regarding the toxicity of spinosad to reptiles or terrestrial-phase amphibians in the EPA or APHIS ecological risk assessments (U.S. EPA/OPP/EFED 2005, 2009a, 2010a, 2011a) and (USDA/APHIS 2003, 2011, 2014), or in the review by Pauli et al. (2000). No other information on the toxicity of spinosad to reptiles or terrestrial-phase amphibians was identified in the open literature. As noted in the EPA risk assessments, the EPA recommends the use of birds as surrogates for reptiles and terrestrial-phase amphibians.

A concern with the use of birds as a surrogate for amphibians involves the permeability of amphibian skin to pesticides and other chemicals. Quaranta et al. (2009) indicate that the skin of the frog *Rana esculenta* is much more permeable than pig skin to several pesticides and that these differences in permeability are consistent with differences in the structure and function of amphibian skin, relative to mammalian skin. In the absence of data, however, the current risk assessment defers to the EPA, and birds are used with reservation as surrogates for reptiles and terrestrial-phase amphibians.

4.1.2.4. Terrestrial Invertebrates

4.1.2.4.1. General Considerations

As discussed in SERA (2014a, Section 4.1.2.4), assays for toxicity to the honeybee are standard EPA requirements for pesticide registration, and acute toxicity data on the honeybee involving oral and contact assays are commonly used as a surrogate for other terrestrial invertebrates. As discussed further below, the literature on the effects of spinosad on terrestrial insects is extensive and covers many different species. Nonetheless, the effects of spinosad on bees are important to the assessment of the potential effects of spinosad on pollinators. All of the product labels for the formulations of spinosad specifically encompassed by the current risk assessment (Table 3) contain relatively standard language on potential effects to pollinators:

> *This product is toxic to bees exposed to treatment for 3 hours following treatment. Do not apply this pesticide to blooming, pollen-shedding or nectar-producing parts of plants if bees may forage on the plants during this time period.*
>
> Product label for Entrust SC

The genesis of the "3 hour" language is discussed further in Section 4.1.2.4.4.2 (Field and Field Simulation Studies).

In terms of practical utility to the risk assessment, the most relevant studies are those for which defined doses in units of mg/kg bw can be determined. These studies are addressed in Section 4.1.2.4.2 (Oral Toxicity) and Section 4.1.2.4.3 (Contact Toxicity). A limitation in these studies is that only relatively few species of terrestrial invertebrates have been assayed. This limitation is addressed with analyses of other toxicity studies using the Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC) system (Section 4.1.2.4.4), efficacy studies from ECOTOX (Section 4.1.2.4.5), and considerations of resistance in insect populations (Section 4.1.2.4.6). While most of the toxicity data on terrestrial invertebrates involve studies on insects and other arthropods, the limited information on earthworms is addressed in Section 4.1.2.4.7.

Mechanistically, spinosyns act on the insect nervous system causing excitation of the neurons, primarily by the stimulation of nicotinic acetylcholine (nAChR) receptors and secondarily by the stimulation of gamma-aminobutyric acid (GABA) gated chloride channels (Barbosa et al. 2015; Shi et al. 2011; Thompson et al. 2000, 2015; U.S. EPA/OPP 2009a, 2012a; U.S. EPA/OPP/EFED 2005, 2011a). In terms of resistance, the spinosyns, including spinosad, are classified as Group 5 nAChR modulators but are considered distinct from other types of nAChR modulators in Group 4 which includes the neonicotinoids (IARC 2015). In worker honeybees, spinosad is associated with both the inhibition of both AChE and ATPase—i.e., an enzyme central to energy metabolism (Rabea et al. 2010). These biochemical mechanisms are associated with gross signs of neurotoxicity that include tremors and involuntary muscle contractions which can lead to neuromuscular fatigue, paralysis, cessation of feeding, and eventually death (e.g., Musser and Shelton 2005; Salgado 1998, Salgado et al. 1998; Thompson et al. 2000).

The sublethal effects of spinosad include impaired flight (Tom et al. 2015) as well as reproductive impairment which is demonstrated in several groups of insects including Lepidoptera (Pineda et al. 2007), Neuroptera (Rimoldi et al. 2012), Diptera (Romi et al. 2006), and Hymenoptera (Schneider et al. 2004; Wang et al. 2012a).

Thompson et al. (2015) note that spinosad …*demonstrates rapid contact and ingestion activity in insects which is unusual for a biological product*. This statement appears to refer to sublethal rather than lethal effects. In terms of lethality, insect death may not occur until several days after initial exposure (Nowak et al. 2001; Thompson et al. 2000). As discussed below and detailed in Appendix 3, marked temporal effects are apparent in some studies on acute toxicity (e.g., Herzog et al. 2002; Schneider et al. 2004).

For most insecticides, toxicity tends to increase with increasing temperatures, and this pattern was observed in grasshoppers (Amarasekare and Edelson 2004). The opposite pattern—i.e., decreasing toxicity with increasing temperature—was observed in houseflies (Diptera, Khan and Akram 2014) and corn borers (Lepidoptera, Musser and Shelton 2005). The available data do not address the effect of temperature on spinosad toxicity to bees.

While not explicitly covered in the current risk assessment, spinosad baits, specifically GF120, are used for the control of fruit flies. Several studies note the avoidance of these bait formulations by some bees, including honeybees (Cabrera-Marin et al. 2015; Gomez-Escobar et al. 2014; Mangan and Moreno 2009), as well as other groups of nontarget insects (Cisneros et al. 2002; Michaud 2003). The avoidance of spinosad baits by bumble bees (Morandin et al. 2005), *Plebeia moureana* (Hymenoptera: Apidae), a species of stingless bee (Sanchez et al. 2012), or some nontarget dipterans (Wang and Messing 2006) is not documented in the available literature. Apart from the GF120 bait formulation, no avoidance of a 480 g/L formulation (i.e., Tracer®) was noted in a controlled laboratory study with *Chelonus insularis*, a nontarget hymenopteran parasitoid.

As discussed further in Section 4.1.2.4.6, several studies address the development of insect resistance to spinosad. Many of these studies note that piperonyl butoxide and other inhibitors of cytochrome P450 synergize the toxicity of spinosad (Bao et al. 2014; Markussen and Kristensen

2012; Scott 1998). The synergism of insecticides by inhibitors of cytochrome P450 suggests that at least some insects metabolize spinosad via cytochrome P450 and that this metabolism is a detoxicification pathway.

4.1.2.4.2. Oral Toxicity

Studies on the oral toxicity of spinosad to terrestrial invertebrates are summarized in Appendix 4: Table A4-1 for honeybees, Table A4-2 for other bees, and Table A4-3 for other terrestrial invertebrates. An overview of the studies for which doses can be expressed in units of mg/kg bw is given in Table 17. Most acute oral and acute contact toxicity studies express doses in units of mg/insect. As with toxicity data on vertebrates, the normalization of toxicity data for insects to units of mg/kg bw is useful for intraspecies comparisons of sensitivity to account for differences in body weights among various species of insects (e.g., Thompson 2015). Most studies on spinosad do not report the body weights of the insects. In order to normalize the doses in units of mg/kg bw, insect body weight data are taken as needed from other publications, as specified in Table 18.

Oral LD_{50} values are available only for bees [Hymenoptera] from the Apidae family—i.e., seven LD_{50} values for the honeybee (*Apis mellifera*) and one LD_{50} value each for a bumblebee (*Bombus terrestris*) and a stingless bee (*Melipona quadrifasciata*). The oral LD_{50} values in honeybees are remarkably consistent with a range of 0.41 to 0.52 mg a.i./kg bw. Based on the review by the European Commission (2006) the toxicity of the NAF-85 formulation (i.e., LD_{50} = 0.42 mg a.i./kg) is not remarkably different from the toxicity of technical grade spinosad. As noted in Table 1, NAF-85 is a 44.2% a.i. formulation. Similarly, Mayes et al. (2003) reports an LD_{50} for the honeybee of 0.50 mg a.i./kg bw for a 480 SC formulation. The similarities between the toxicity values for technical grade spinosad and spinosad formulations suggest the inerts in the formulations do not have a substantial impact of the toxicity of the formulations to honeybees.

Based on assays using the 480 SC formulation of spinosad, the honeybee appears to be more sensitive by a factor of about 3, compared with either the bumble bee [$0.50 \div 0.13 \approx 3.84$] or the stingless bee (*Melipona quadrifasciata*) [$0.50 \div 0.15 \approx 3.33$]. As discussed further in Section 4.1.2.4.3 (Contact Toxicity), the apparently greater sensitivity of the bumble bee relative to the honeybee following oral exposure is not reflected in the contact toxicity studies.

In addition to the acute oral toxicity studies on adult bees, discussed above, Barbosa et al. (2015) conducted a 20-day oral toxicity study on larvae of another species of stingless bee, *Melipona quadrifasciata*. This study also used a 480 g a.i./L SC formulation of spinosad. As summarized in Appendix 3, Table A3-2, signs of toxicity in larvae included decreased survival, decreased pupal body weights, and increases in the incidence of malformations at doses of about 0.059 mg a.i./kg bw/day and higher. No signs of larval toxicity were observed at or below doses of 0.012 mg a.i./kg bw/day. In newly emerged adults, however, abnormal walking activity was observed at doses of about 0.0012 mg a.i./kg bw/day or higher. The NOAEL for changes in walking activity was about 0.00059 mg a.i./kg bw/day. The publication does not provide an estimate of the LD_{50} but does provide a survival probability plot (Barbosa et al. 2015, Figure 1B). Based on this plot, 50% mortality occurred at about 18 days after dosing and at reported doses of about 0.059 to 0.12 mg a.i./kg bw/day. As discussed above and summarized in Table 17, these doses

are only moderately below the acute oral LD_{50} of 0.15 mg a.i./kg bw for *Melipona quadrifasciata* (Tom et al. 2015).

In addition to the studies on bees, Table 17 also includes LD_{50} values for the American cockroach (*Periplaneta americana*) and the tobacco budworm (*Heliothis virescens*) larvae from the study by Salgado (1998) following abdominal injection of spinosyn A. As discussed in Section 2, spinosyn A is the major component of spinosad. While these data are not directly comparable to oral toxicity data, the relatively high LD_{50} values in the cockroach (i.e., 1.1 and 2.7 mg/kg bw) suggest that cockroaches may be somewhat less sensitive than bees. The relatively low LD_{50} value of 0.23 mg/kg bw in tobacco budworm larvae is intermediate between the low oral LD_{50} values for the bumblebee and stingless bee and the somewhat higher LD_{50} values for honeybees.

4.1.2.4.3. Contact Toxicity

Studies on the contact toxicity of spinosad to terrestrial invertebrates are summarized in Appendix 4: Table A4-1 for honeybees, Table A4-2 for other bees and Table A4-3 for other terrestrial invertebrates. An overview of the studies for which doses can be expressed in units of mg/kg bw is given in Table 19. As with the corresponding table on oral toxicity, the insect body weight data used to normalize the doses to units of mg/kg bw are given in Table 18. The contact studies summarized in Table 19 involve the use of a micropipette to deposit a known amount of spinosad onto the body (typically the thorax) of the insect (e.g., U.S. EPA/OCSPP 2012b).

4.1.2.4.3.1. Contact Toxicity to Bees

Based on the contact LD_{50} of 2.9 ng (≈0.025 mg/kg bw) from Hoxter et al. (1992), the EPA classifies technical grade spinosad as …*highly toxic toward honey bees* (U.S. EPA/OPP/EFED 2011a, p. 35). As summarized in Table 19, however, the variability in the toxicity data for technical grade spinosad in *Apis mellifera* is much greater than the corresponding values for oral toxicity—i.e., LD_{50} values of about 0.5 mg/kg bw (Section 4.1.2.4.1). The relatively low LD_{50} of 0.025 mg/kg bw from Hoxter et al. (1992) is well documented and is supported by an LD_{50} of about 0.031 mg/kg bw from the European Commission (2006). Details of the study used by the European Commission (2006) are not available. Three other contact LD_{50} values for technical grade spinosad in *Apis mellifera* range from 0.34 to 0.67 mg/kg bw and are much closer to the oral LD_{50} values. As discussed further in Section 4.1.2.4.6 (Resistance), differences in sensitivity of about a factor of 10 might be expected from organisms taken from different populations in the field. Whether or not this might account for the differences in the LD_{50} values for technical grade spinosad in *Apis mellifera* cannot be determined from the available data.

Another difference between the oral and contact LD_{50} values for *Apis mellifera* involves the toxicity of formulations. As discussed in Section 4.1.2.4.2 and summarized in Table 17, the oral LD_{50} values for technical grade spinosad are similar to the LD_{50} values for formulations. Based on the LD_{50} values for topical applications, a 1.6% wettable power (WP) formulation appears to be comparable in toxicity to technical grade spinosad, and the 480 suspension concentrate (SC) formulation (i.e., 480 g/L) appears to be substantially less toxic than technical grade spinosad. Consistent with data in mammals (Section 3.1.14.1), the data for the honeybees do not suggest that other ingredients (i.e., inerts) in spinosad formulations contribute substantially to toxicity.

In addition to studies on honeybees, data are available on other bee species including the bumble bee (*Bombus terrestris*, Apidae), the alfalfa leafcutter bee (*Megachile rotundata*, Megachilidae), and the alkali bee (*Nomia melanderi*, Halictidae). The study by Mayer et al. (2001) provides data on the latter two species as well as the honeybee, and these may be considered essentially matched bioassays—i.e., conducted using the same methods by the same set of investigators. Based on the data from Mayer et al. (2001), the honeybee appears to be the most sensitive bee species with an LD_{50} of 0.61 mg/kg bw. The alkali bee (*Nomia melanderi*, Halictidae) is somewhat less sensitive with an LD_{50} of 0.76 mg/kg bw. The alfalfa leafcutter bee (*Megachile rotundata*, Megachilidae), with an LD_{50} of 1.9 mg/kg bw, appears to be less sensitive than the honey bee by a factor of about 3 [1.9 mg/kg bw ÷ 0.61 mg/kg bw ≈ 3.1148]. At specified in Appendix 3, however, the 95% confidence intervals for the three LD_{50} values overlap – i.e., the apparent differences in sensitivities may not be statistically significant.

The data on the bumble bee come from the review by Mayes et al. (2003) summarizing a study conducted by Dow AgroSciences. As discussed in Section 4.1.2.4.2 (oral toxicity) and summarized in Table 17, the bumble bee appears to be more sensitive than the honey bee by about a factor of 3 based on oral LD_{50} values. Based on contact assays with a 480 SC formulation, however, bumble bees appear to substantially less sensitive than honey bees. Taking the highest LD_{50} for an 480 SC formulation, the magnitude of the difference in sensitivity is about a factor of 8 [65 mg/kg bw (bumble bee) ÷ 8.5 mg/kg bw (honeybee) ≈ 7.6].

While the discussion of relative sensitivities focuses on doses that can be expressed in units of mg/kg bw, the studies by Bailey et al. (2005) and Scott-Dupree et al. (2009) on direct spray applications (i.e., LC_{50} values expressed in units of mg/L) can be used to elaborate on differences in sensitivities among bees. The papers by Bailey et al. (2005) and Scott-Dupree et al. (2009) are from the same group of investigators using the same direct spray exposures. These two studies are summarized in Table 20. Consistent with the standard micropipette studies discussed above, the bumble bee appears to be less sensitive than the honeybee by a factor of about 4 [89.5 mg/L (bumblebee) ÷ 22 mg/L (honeybee) ≈ 4.07]. Unlike the case with the topical applications from Mayer et al. (2001) in which the leafcutter bee was less sensitive than the honeybee by a factor of about 3 [1.9 mg/kg bw ÷ 0.61 mg/kg bw ≈ 3.1148], the direct spray assay from Scott-Dupree et al. (2009) suggests that the leafcutter bee is more sensitive than the honeybee by a factor of about 2 [12.5 mg/L (leafcutter bee) ÷ 22 mg/L (honeybee) ≈ 0.56; 1 ÷ 0.56 ≈ 1.78].

4.1.2.4.3.2. Contact Toxicity to Other Terrestrial Insects

Differences in toxicity among various groups of organisms are a fundamental concern in any ecological risk assessment. In some cases, differences among species may systematically relate to body weight even when dose is scaled to units of mg/kg bw (i.e., allometric relationships as discussed by Sample and Arenal 1999). Based on the contact LD_{50} values (in units of mg/kg bw) from Table 19 and as illustrated in Figure 6, this does not appear to be the case for differences in the toxicity of spinosad in insects. Over a relatively wide range of body weights (i.e., about 2 mg to 30 mg), no systematic differences in sensitivity are apparent for three orders of Diptera, including Muscidae (house flies), Culicidae (mosquitoes), and Tephritidae (fruit flies). As discussed in Section 4.1.2.4.3.1, bumblebees [Hymenoptera: Aphidae, tribe Bombini] appear to be more tolerant than other hymenopterans to spinosad. No other substantial trends in toxicity are apparent for the other hymenopterans over a range of body weights that exceeds an order of

magnitude—i.e., about 8 mg for *Nomia melander* to 116 mg for *Apis mellifera*. Two data points are available for the Lepidoptera—i.e., adult *Helicoverpa armigeram* from the study by Achaleke et al. (2009) and *Manduca sexta* larvae from the study by Herzog et al. (2002). Given that only two data points for Lepidoptera are available and that these data points involve different life stages and different families, generalizations concerning sensitivity and body weights are not warranted.

Another approach to looking at differences in sensitivity among different groups of organisms involves *sensitivity distributions* (e.g., Awkerman et al. 2008; Posthuma et al. 2002). The quantitative use of species sensitivity distributions in risk assessment is discussed in detail by EPA (https://www3.epa.gov/caddis/da_advanced_2.html). While typically applied at the level of species, the honeybee (*Apis mellifera*) is the only species for which several bioassays are available. Thus, as an exploratory effort, sensitivity distributions are applied at the level of insect order in Figure 7. Again because of limitations in the number of data points available within the different orders, only Hymenoptera, Diptera, and Lepidoptera are included in Figure 7. Within each these orders, the individual values for the cumulative frequency (plotted on the y-axis of Figure 7) are based on the following equation:

$$Freq_i = \frac{i - 0.5}{N} \quad (5)$$

where $Freq_i$ is the cumulative frequency for the i^{th} value and N is the number of values in the data set. As detailed by Posthuma et al. (2002), the development of sensitivity distributions involves an ordered ranking of the available toxicity values (i.e., lowest to highest) in which "*i*" in the above equation is the ordinal rank – i.e., 1st, 2nd, 3rd, and so on. The 0.5 constant in the above equation is factor to adjust for the ordinal ranking to approximate a midpoint. For example, thirteen LD_{50} values are available for the hymenopterans (Table 18). The lowest value is 0.025 mg/kg bw, the LD_{50} for *Aphis mellifera* from the study by Hoxter et al. (1992). The frequency for this value is about 0.038462 [(1-0.5) ÷ 13]. The second lowest LD_{50} for hymenopterans is 0.031 mg/kg bw, also for *Aphis mellifera* (European Commission 2006). This frequency is about 0.115385 [(2-0.5) ÷ 13]. The x-axis in Figure 7 represents the LD_{50} value corresponding to the frequency. The x-axis uses a logarithmic scale under the standard assumption that LD_{50} values for different chemicals or different groups of organisms have a lognormal distribution.

As illustrated in Figure 7, no remarkable differences in sensitivity are apparent among the three orders of insects for the left-most points (i.e., areas of greater sensitivity). The data on Lepidoptera are limited to only two points. Nonetheless, the similarities among the Hymenoptera, Diptera, and Lepidoptera are striking in terms of similarities among presumably sensitive species in these orders of insects.

The upper and right-most three points in Figure 7 for the Hymenoptera, however, appear to be somewhat right-shifted in that these points appear to reflect an atypical tolerance to spinosad. This is particularly true for the right-most point which is for the bumblebee (*Bombus terrestris*, Apidae). This point is from the study by Mayes et al. (2003) using a 480 SC formulation. As discussed in Section 4.1.2.4.3.1, the bumblebee appears to be substantially more tolerant to this

spinosad formulation, relative to comparable data on *Apis mellifera* (Table 19); moreover, this difference appears to be statistically significant based on the direct spray bioassays (Table 20). The other two right-shifted points in Figure 7 are from bioassays with the 480 SC formulation in *Aphis mellifera* (i.e., the studies by Mayer et al. 2003 and Miles 2003), as summarized in Table 19. As discussed in Section 4.1.2.4.3.1, the 480 SC formulation appears to be less toxic than technical grade spinosad. Thus, the apparent tolerance of bumblebee – i.e., the most right-shifted point in Figure 7, may be due to the use of a less-toxic formulation rather than to true differences in species sensitivity.

4.1.2.4.4. Other Toxicity Studies

4.1.2.4.4.1. IOBC Classifications

In addition to studies in which exposures can be characterized as doses in units of mg/kg bw, there is a large and diverse literature on the toxicity of spinosad to terrestrial insects and other arthropods regarding various forms of contact or residual exposures (e.g., leaf dip assays, immersion assays, and various assays for sublethal effects). It is beyond the scope of the current risk assessment to discuss all of these studies in detail. Nonetheless, two detailed reviews (Williams et al. 2003b; Miles and Eelen 2006) summarize many of the toxicity studies to diverse groups of nontarget organisms using study classification systems developed by International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC). Similar to the EPA ranking system discussed in Section 3.1.4.1, the IOBC system classifies the results of studies using rankings of 1 (less harmful) to 4 (most harmful) (Boller et al. 2005). As summarized in Table 21, the IOBC rankings were applied to laboratory studies (n=104) by Williams et al. (2003b) and to laboratory, semi-field, and field studies (n=299) by Miles and Eelen 2006. The last column of Table 21 gives a weighted score (S) for each group of invertebrates which is calculated as:

$$S = \sum_{i=1}^{4} i \times \frac{n_i}{N} \quad (6)$$

Where i is the score (1, 2, 3, or 4), n_i is the number of studies for the group (e.g., Hemiptera) with a score of i, and N is the total number of studies for the group. For example, the first data row in Table 21 (Hemiptera from Williams et al. 2003b) gives a weighted score of 1.36 based on eight studies in Category 1, two studies in Category 2, one study in Category 3, and no studies in Category 4. This score is calculated as (8 x 1) + (2 x 2) + (1 x 3) + (0 x 4)) ÷ 11. Note that the weighted score is not part of the IOBC scheme but is used in the current risk assessment to facilitate visualization of the data in Figure 8 which gives the weighted scores for each group in Table 21 separately for the analyses by Williams et al. (2003b) [upper portion of Figure 8] and Miles and Eelen (2006) [lower portion of Figure 8].

Parasitic wasps (Hymenoptera) are rated as the most sensitive group of terrestrial insects by both Williams et al. (2003b) and Miles and Eelen (2006). This designation is consistent with the more recent review by Biondi et al. (2012) as well as several other studies on the sensitivity of hymenopteran wasps and other hymenopteran parasitoids (e.g., Beloti et al. 2015; Biondi et al. 2013; de Freitas Bueno et al. 2008; Liu and Zhang 2012). In addition to acute lethality, as discussed in Section 4.1.2.4.1, the sublethal toxicity of spinosad involves adverse effects on reproduction, and several studies on Hymenoptera demonstrate adverse effects on reproductive parameters and longevity (Beloti et al. 2015; Liu and Zhang 2012; Penagos et al. 2005; Schneider et al. 2004; Wang et al. 2012a). A few matched studies involving multiple orders of

insects also demonstrate that spinosad is more toxic to Hymenoptera than to other orders of insects (Clevland et al. 2002b; Jones et al. 2005; Pietrantonio and Benedict 1999; Schoonover and Larson 1995).

The only inconsistency between the rankings from Williams et al. (2003b) and Miles and Eelen (2006) involves the arthropods in the subclass Acari (mites and ticks). This inconsistency is relatively trivial given that Williams et al. (2003b) covered only four studies on the Arcari and the more recent analysis by Miles and Eelen (2006) covered 40 studies. The weight-of-evidence suggests that some mites may be highly sensitive (e.g., *Neoseiulus fallacis* in the study by Villanueva and Walgenbach 2005) but that most mites are less sensitive than wasps to spinosad. The only other groups that appear to be highly sensitive to spinosad are the Dermaptera (earwigs) and Thysanoptera (thrips). As with the Acari in the analysis by Williams et al. (2003b), however, the high composite scores are based on only a few studies, including three studies on Dermaptera in the analysis by Williams et al. (2003b) and two studies on Thysanoptera in the analysis by Miles and Eelen (2006). Nonetheless, as discussed further in Section 4.1.2.4.7 (resistance), spinosad is used extensively for the control of thrips (U.S. EPA/OPP/EFED 2011a), and the apparent high sensitivity of thrips to spinosad is probably not an artifact of small sample size. While the effects of spinosad on Dermaptera are not extensively documented, Cisneros et al. (2002) and Redoan et al. (2013) note that spinosad is detrimental to *Doru* species (Dermaptera: Forficulidae), predators on lepidopteran pests. Alston and Tebeau (2011) recommend spinosad (specifically the Success and Entrust formulations) in discussing methods for the control of the European earwig (*Forficula auricularia*, Dermaptera: Forficulidae), which may be viewed as a pest species on some crops. While this recommendation reinforces the assessment that Dermaptera may be an insect order sensitive to spinosad, the specimen product labels for Entrust SC® and Success® are not specifically labelled for the control of earwigs.

The application of the IOBC system by Williams et al. (2003b) and Miles and Eelen (2006) do not specifically address bees (several orders of Hymenoptera) and Lepidoptera. Field studies with bees are discussed in the following subsection. As discussed in Section 4.1.2.4.5, spinosad is highly toxic to and used to control lepidopteran pests, as indicated by the large number of efficacy studies.

4.1.2.4.4.2. Field and Field Simulation Studies (Bees)

Field and field simulation studies involving the exposure of bees to spinosad are summarized in Appendix 3, Table A3-4. Most of the field and field simulation studies given in Appendix 3 are taken from the detailed review by Mayes et al. (2003) of unpublished studies conducted by Dow AgroSciences. Mayes et al. (2003) identify the specific unpublished studies, and the study designations are given in in Appendix 3, Table A3-4, for the sake of clarity. Because these studies were not available for the conduct of the current Forest Service risk assessment, the studies designated in the review by Mayes et al. (2003) are not included in Section 5 (list of citations) of the current risk assessment. Some publications from the primary literature are also available, including Burns et al. (2001), Morandin et al. 2005, and Sanchez et al. 2012. The study by Burns et al. (2001) was conducted jointly by the USDA in cooperation with Dow AgroSciences. The other two studies were conducted as private organizations—i.e., a research institute in Mexico (Sanchez et al. 2012) and a university in Canada (Morandin et al. 2005).

The study by Sanchez et al. (2012) is a field simulation study involving extremely high concentrations of spinosad in sucrose (i.e., 10 to 80 mg a.i./L) in assays of a stingless bee, *Plebeia moureana*. As discussed further in Section 4.2.3.3 (Nectar Exposures Involving Honeybees), nectar concentrations of 10 to 80 mg a.i./L are far greater than concentrations of spinosad in nectar that might be expected in Forest Service applications. Sanchez et al. (2012) indicate that there was no adverse effect on *Plebeia moureana*. As discussed in Section 4.1.2.4.2 and summarized in Table 17, another species of stingless bee (*Melipona quadrifasciata*) appears to be somewhat more sensitive than the honeybee is to spinosad. While not explicitly assessing the sensitivity of *Plebeia moureana* to spinosad, the study by Sanchez et al. (2012) suggests that *Plebeia moureana* may be relatively insensitive to spinosad.

The open literature study by Burns et al. (2001) used low application rates of up to about 0.0014 lb a.i./acre in efficacy tests for fruit fly control. No adverse effects were observed in foraging bees. This study is considered below in conjunction with several other low application rate studies noting no adverse effects in bees.

The open literature study by Morandin et al. (2005) is a field simulation study in which bumblebee (*Bombus impatiens*) colonies were treated with spinosad-contaminated pollen. While exposures are expressed as concentrations of spinosad in pollen (in units of mg a.i./kg pollen), Morandin et al. (2005) provide estimates of application rates that might be associated with the concentrations of pollen used in the study. Despite some uncertainty with the usefulness of this study, the observations by Morandin et al. (2005) are discussed below with other field or field simulation studies summarized in Mayes et al. (2003).

An overview of the field and field simulation studies is given in Table 22. Most of the studies summarized in Appendix 3, Table A3-4 express exposure in units of g a.i./ha. In Table A3-4, units in lb a.i./acre are given in brackets [] using the conversion factor of 0.892 $_{\text{lb/acre per kg/ha}}$ with rounding to two significant figures. In Table 22, only units of lb a.i./acre are used. Note that Table 22 does not include three greenhouse studies which are summarized at the start of Appendix 3, Table A3-4. All three greenhouse studies are unpublished reports summarized in Mayes et al. (2003). The two studies attributed to Kaneshi (200a,b) note adverse effects in honeybees and bumblebees at relatively low application rates of ≈0.089 lb a.i./acre for honeybees and 0.11 lb a.i./acre for bumblebees.

The greenhouse studies are not included in Table 22 because they are not typical of exposures that would be used in Forest Service programs. Furthermore, as summarized in Table 22, many field and field simulation studies indicate that adverse effects are not likely to occur in bees at application rates of up to 0.16 lb a.i./acre. The specific studies include Burns et al. (2001) as well as the following studies cited by Mayes et al. 2003 are: Forey 1999, Kirkland 1999 (low application rate), Kransfelder 1999, Mayer 1999, Palmer and Krueger 1997, Taylor and Goodwin 2000, and Vinall 2000.

As discussed in Section 2, the typical application rate for Forest Service uses will be about 0.225 lb a.i./acre. As summarized in Table 22, this application rate is somewhat above rates associated with incidental although statistically insignificant increases in mortality in a field study (0.19 lb

a.i./acre) and transient effects on foraging (0.2 lb a.i./acre). At substantially higher application rates in the range of 0.48 to 0.71 lb a.i./acre, effects on colony health (i.e., reduced brood development and worker mortality) have been noted. These field and field simulation studies are discussed further in the risk characterization for bees (Section 4.4.2.4.3).

While contact toxicity (4.1.2.4.3.1) is used directly in the current risk assessment to characterize the risks associated with direct spray, residual contact toxicity is a concern—i.e., the contact of a bee with contaminated vegetation following the foliar application of spinosad. As noted in Section 4.1.2.4.1, the product labels specify that spinosad applications may be … *toxic to bees exposed to treatment for 3 hours following treatment*. This language appears to reflect field simulation studies (summarized in Appendix 3, Table A3-4) in which no signs of acute toxicity were observed in bees following exposure to vegetation that was treated 3 hours prior to exposing the bees to the vegetation (Mayes et al. 2003 citing unpublished studies by Kransfelder 1999; Palmer and Krueger 1997) and the lack of effects on mortality or brood development in honeybee colonies following exposure to treated vegetation that had been covered for 3 hours—i.e., effectively preventing direct exposure to the contaminated vegetation prior to drying of the applied solution (Mayes et al. 2003 citing unpublished study by Mayer 1999). The reduced toxicity to bees (i.e., no signs of overt toxicity) was also observed following applications of spinosad conducted in the evening when bees are not actively foraging—i.e., Mayes et al. (2003) citing unpublished studies by Taylor and Goodwin (2000) and Goodwin and Haine (1998). Lastly, Mayer et al. (2001) conducted residual contact assays in alfalfa leafcutter bees and alkali bees noted generally lower mortality using vegetation assayed at 8 hours after treatment relative to 2 hour after treatment.

These field and field simulation studies are consistent with studies summarized in US EPA/OPP/EFED (2011a) indicating that the toxicity of spinosad residues on vegetation is substantially reduced by a post-application period of 3 hours prior to exposing bees to the vegetation (i.e., MRID 45007701 and MRID 45007702 as summarized in Appendix 3, Table A3-1). The impact of reducing exposures to bees during or for a period of time after application is an important consideration in the risk characterization for bees (Section 4.4.2.4.1).

4.1.2.4.5. Efficacy Studies

Efficacy studies are not typically detailed or otherwise used in Forest Service risk assessments. As noted in Section 4.1.2.4.3.2 and illustrated on Figure 7, Lepidoptera appear to be about as sensitive as Hymenoptera are to spinosad; however, this observation is based only on two data points for the Lepidoptera. Similarly, as discussed in 4.1.2.4.4.1 and illustrated in Figure 8, the analyses of numerous laboratory and field studies using the IOBC system do not include Lepidoptera, which are target rather than nontarget species. To elaborate on the sensitivity of lepidopteran species (moths and butterflies) to spinosad, a search was conducted of EPA's ECOTOX database to identify LOAELs expressed in units of application rate (i.e., lb a.i./acre) for Lepidoptera as well as other orders of insects. As summarized in Table 23, this search yielded records dominated by Lepidoptera (a common target species for spinosad) but also several records for Coleoptera (n=4), Hemiptera (n=3), and Hymenoptera (n=5) and single records for Diptera and Orthoptera. These data are illustrated in Figure 9 using sensitivity distributions as discussed in Section 4.1.2.4.3.2.

Because of the small number of points for non-lepidopteran orders of insects, sensitivities relative to lepidopterans can be assessed only crudely. Nonetheless, and consistent with the sensitivity distributions for contact LD_{50} values, the sensitivity of Lepidoptera appears to be similar to that of Hymenoptera at least at the lower and upper bounds of the application rates involving Hymenoptera. Consistent with the application of the IOBC scores (Table 21, Figure 8), the Hemiptera appear to be substantially less sensitive relative to the Lepidoptera—i.e., the points for Hemiptera in Figure 9 are right-shifted from points for Lepidoptera. The tolerance of Hemiptera to spinosad is noted in the open literature (Baur et al. 2003; Eelen et al. 2006; Elzen and Elzen 1999; Martinou et al. 2014); furthermore, a matched direct spray assay of a hymenopteran (*Encarsia formosa*, Aphelinidae) and hemipteran (*Orius insidiosus*, Anthocoridae) notes the greater tolerance of the Hemiptera (Jones et al. 2005). While spinosad is classified as highly toxic to *Orius insidiosus* (Hemiptera: Anthocoridae) in petri dish assays, toxic effects are not documented in the more realistic exposures in field and greenhouse assays (Studebaker and Kring 2003, Table 1).

The data on Coleoptera are based on only two studies, and the data on Diptera and Orthoptera are based on only a single study each. These data are not sufficient to assess sensitivities relative to Lepidoptera.

4.1.2.4.6. Insect Resistance

The spinosyns (i.e., both spinosad and spinetoram) are classified by the IRAC Resistance Action Committee as Group 5: Nicotinic acetylcholine receptor (nAChR) allosteric modulators (IRAC 2016). This mode of action classification is unique to the spinosyns. A variety of related nAChR competitive modulators (rather than allosteric) modulators—e.g., neonicotinoids and sulfoximines—are classified as a mechanistically distinct group from the spinosyns in terms of mechanisms for resistance.

Resistance often has an impact on efficacy. As noted in Section 4.1.2.4.5, however, efficacy is not a focus of the current risk assessment. Nonetheless, the potential for resistance in populations of the same or closely related species complicates the current risk assessment in that resistance (or more generally variability in sensitivity among different populations) confounds the assessment of systematic differences in sensitivity among different groups of terrestrial invertebrates. For example, as discussed in Section 4.1.2.4.3 and summarized in Table 19, differences in contact LD_{50} values vary by a factor of about 12 [3.97 mg/kg bw ÷ 0.33 mg/kg bw ≈ 12.03] for Diptera: Tephritidae and by a factor of about 27 [0.67 mg/kg bw ÷ 0.025 mg/kg bw = 26.8] for *Apis mellifera*.

Resistance studies in four orders of target insects (i.e., Coleoptera, Diptera, Lepidoptera, and Thysanoptera) are summarized in Table 24. Resistance is typically quantified as resistance factors or ratios—i.e., the ratio of a dose associated with a defined response (e.g., LC_{50}) in resistant populations to the dose associated with the same response in a sensitive population. Note that some of the resistance factors given in Table 24 are less than one. In all cases, these are examples of the investigators calculating the resistance factor as the ratio of the toxicity value for a field population to the corresponding toxicity value for a laboratory population. Thus, resistance factors of less than one simply indicate that the field population is more sensitive than the laboratory reference population.

As noted in Table 22, resistance factors for spinosad range up to nearly 3 million (i.e., *Frankliniella occidentalis* in the study by Bielza et al. 2007). This and several other studies summarized in Table 22 involve the artificial generation of resistance developed by subjecting multiple generations of insect populations to lethal doses (i.e., LD_{50} to LD_{90}) of spinosad and breeding subsequent generations with the survivors of the bioassays. These types of exposures are not likely to occur in the environment, and the very high resistance factors may be viewed as physiological maximum potential resistance factors. The studies using artificial resistance pressure are given in bold font in Table 22.

Other types of studies summarized in Table 22 involve simpler comparisons of field populations (presumably subject to selection pressures in the normal use of spinosad) to laboratory populations not subject to artificial selection pressure (e.g., Huang et al. 2004; Hsu et al. 2012a). Most of the studies that focus on natural field populations of resistant insects note only moderate resistance factors in the range of about 0.6 to 13 (Achaleke et al. 2009; Huang et al. 2004; Hsu et al. 2012a; Scott 1998; Zhang et al. 2014). In some instances, the low reported resistance factors may reflect simple variability in field populations rather than true resistance (i.e., Huang et al. 2004). Some field populations of Diptera, however, have much greater resistance factors—i.e., *Musca domestica* from the study by (Gao et al. 2007a) and some populations of *Drosophila melanogaster* from the study by Rinkevich and Scott (2013). In addition, resistance factors of up to nearly 2000 were observed in field populations of thrips (*Thrips palmi*) not subject to artificial selection pressure (Bao et al. 2014).

The mechanisms of resistance are unclear. Some studies associate resistance at least partially with an increased detoxification by cytochrome P450 isozymes (Bao et al. 2014; Markussen and Kristensen 2012; Sayyed et al. 2008). Several other studies note no apparent relationship of P450 activity with resistance and suggest that the primary mechanism of resistance involves changes in the underlying receptor site (Bielza et al. 2007; Hsu et al. 2012b; Gao et al. 2007a; Shi et al. 2011; Campos et al. 2014). Many studies indicate that resistance is a stable trait in the absence of cross-breeding (Bielza et al. 2007) but appears to be a recessive trait if the resistant populations crossbreed with non-resistant populations (Campos et al. 2014; Hou et al. 2014). According to several studies, spinosad resistance may be a recessive trait due to increased energy requirements, alterations in immune function, or delayed developmental effects associated with the resistance to spinosad (Sayyed et al. 2008; Sagri et al. 2014).

Consistent with the presumably unique mechanism of resistance, at least four studies note that resistance to spinosad is not associated with cross-resistance to other pesticides and resistance to other pesticides is not associated with cross-resistance to spinosyns (Achaleke et al. 2009; Bielza et al. 2007; Hsu and Feng 2006; Hussain et al. 2009).

Resistance to spinosad is noted also in aquatic assays of mosquito larvae (Khan et al. 2011a; Liu et al. 2004a,b), as discussed further in Section 4.1.3.3.

4.1.2.4.7. Earthworms

The toxicity of spinosad to earthworms is not addressed in the open literature, including standard compendia of earthworm toxicity studies (i.e., Edwards and Bohlen 1992; Potter et al. 1990, 1994; Wang et al. 2012).

The earthworm is the standard test species used by the EPA to assess the potential hazards to soil invertebrates (U.S. EPA/OCSPP 2012b). The most recent EPA ecological risk assessment includes a brief summary of a 14-day soil bioassay in *Eisenia foetida* in which a concentration of 970 mg a.i./kg soil was not associated with signs of toxicity based on biomass (U.S. EPA/OPP/EFED 2011a, p. 35, MRID 43414548). As summarized in Table 5, the toxicity value of 970 mg a.i./kg soil is given on the Material Safety Data Sheets for spinosad as an indefinite LC_{50} (i.e., LC_{50} > 970 mg a.i./kg soil).

A review of spinosad by the European Commission (2006, p. 31) notes an acute LD_{50} for NAF-85 (i.e., a 44.2% a.i. formulation as noted in Table 1) of >458 mg a.i./kg soil and an acute LC_{50} of >500 mg/kg soil for N-demethylated spinosyn D. In addition, the review by the European Commission notes a reproductive NOEC of >2700 g a.i./ha (≈2.4 lb a.i./acre) for NAF-85 and a reproductive NOEC for N-demethylated spinosyn D of >964 mg/kg soil. Details of these unpublished studies are not given in European Commission (2006) review.

While HQs for earthworms are not typically derived in Forest Service risk assessments, the scant data on earthworms are considered further in the dose-response assessment (Section 4.3.2.5.4) and risk characterization (Section 4.4.2.4.4).

4.1.2.5. Terrestrial Plants (Macrophytes)

Studies concerning the toxicity of spinosad to terrestrial plants are summarized in Appendix 4. These studies are limited to standard Tier 1 (i.e., single dose) studies on vegetative vigor (Table A4-1) and seedling emergence (Table A4-2). For herbicides, the EPA generally requires relatively sophisticated Tier II bioassays on plants. For insecticides applied to plants, much simpler Tier 1 (i.e., single limit dose) studies are sometimes required. Up until recently, the U.S. EPA judged that the available Tier 1 studies on terrestrial plants are adequate and that additional Tier 2 testing would not be required (e.g., U.S. EPA/OPP/EFED 2009a, p. 47; U.S. EPA/OPP/EFED 2010a, p. 13).

As specified in Appendix 4, the Tier 1 studies were conducted at an application rate of 0.5 lb a.i./acre. In the EPA's more recent assessment for the registration review of spinosad, however, the EPA notes that the available Tier 1 studies were not conducted at the maximum registered application rate (≈0.8 lb a.i./acre). Thus, the EPA is requiring an …*acceptable tier I study is needed that tests the effects of the maximum labeled application rate to terrestrial plants* (U.S. EPA/OPP/EFED 2011a, p. 56). Note that the EPA is not requiring Tier 2 testing.

This data reservation is noted for the sake of transparency but does not impact the current Forest Service risk assessment. As discussed in Section 2, the maximum seasonal application rate proposed by the Forest Service is 0.45 lb a.i./acre—i.e., two applications of 0.225 lb a.i./acre.

As discussed in previous sections, spinosad has been applied to many species of plants for the control of insect pests with no apparent adverse effects. In the absence of documented phytotoxicity and given that the available Tier 1 studies are above the application rates proposed by the Forest Service, toxicity to terrestrial vegetation is not identified as a potential hazard.

4.1.2.6. Terrestrial Microorganisms

The U.S. EPA/OPP does not typically require bioassays for microbial toxicity, and the potential effects of spinosad on terrestrial microorganisms are not addressed in the available EPA risk assessments (Section 1.1). The EPA does have a protocol for a 12-week soil-core microcosm assay; however, this test is focused on functional changes to soil, based on observations of plant growth. Assays for effects on microorganisms are optional (U.S. EPA/OCSPP 2012a). This assay does not appear to have been conducted with spinosad.

The European Commission (2006, p. 31) provides a brief summary of unpublished studies on the toxicity of spinosad to soil microorganisms, which indicates that spinosad, at a soil concentration of 7.2 mg/kg soil, caused a transient decrease (-55%) in soil nitrification after 15 days but that the effect was <25% at "*test termination*" (not otherwise specified). No substantial effects were noted on carbon mineralization at 7.2 mg/kg and no effects on either nitrogen or carbon mineralization were noted at 0.72 mg/kg soil. N-demethylated spinosyn D caused no effects on nitrogen or carbon mineralization at concentrations of 0.3855 or 1.928 mg/kg soil. As discussed further in the risk characterization (Section 4.4.2.6), the anticipated levels of spinosad in soil following one or two applications at 0.225 lb a.i./acre are below the 0.72 mg/kg NOAEC noted in European Commission (2006).

4.1.3. Aquatic Organisms

4.1.3.1. Fish

4.1.3.1.1. Acute Toxicity

Studies on the acute toxicity of spinosad and spinosad formulations in fish are summarized in Appendix 5, Table A5-1. The U.S. EPA typically uses 96-hour LC_{50} values in fish to assess the potential for acute risks to fish. Based on the 96-hour LC_{50} of 5.94 mg a.i./L in bluegill sunfish, the EPA classifies spinosad as moderately toxic to bluegill sunfish (MRID 43414534). Based on the 96-hour LC_{50} of 30 mg a.i./L in rainbow trout, the EPA classifies spinosad as slightly toxic to trout (MRID 43414534) (MRID 43444103) (U.S. EPA/OPP/EFED 2011a, p. 30). The lowest reported 96-hour LC_{50} is 4 mg a.i./L in carp. Carp is not a standard test species used by EPA. The LC_{50} in carp is taken from the review by the European Commission (2006). Details of this study are not given on the European Commission review. As summarized in Table 5, the Material Safety Data Sheets for the representative formulations of spinosad considered in the current risk assessment specify LC_{50} values in fish as 0.1 to 1 mg/L. These toxicity values are not included in the EPA ecological risk assessments on spinosad.

Two indefinite acute LC_{50} values are available on spinosad formulations—i.e., a 96-hour LC_{50} of >49 mg a.i./L for carp (European Commission 2006) and a 96-hour LC_{50} of >500 mg/L for Coho salmon from Deardorff and Start (2009). Based on the data on carp for both technical grade spinosad and the NAF-85 formulation, it does not appear that other ingredients in the formulation contribute to the toxicity of spinosad.

The effects of many pesticides and other chemicals include general signs of oxidative stress typically characterized by an increase in free radical production and other reactive oxygen species leading to increased lipid peroxidation, generalized tissue damage, cell death, and depletion of endogenous antioxidants such as glutathione. General oxidative damage is a

common effect noted in mammals (Abdollahi et al. 2004; Agrawal and Sharma 2010) as well as fish (Slaninov et al. 2009; Stoliar and Lushchak 2012). As summarized in Appendix 5, Table A3-1, Pine and Uner (2013, 2014) observed biochemical markers indicative of oxidative stress in tilapia at spinosad concentrations of 25 mg a.i./L or greater using Laser, a 480 g a.i./L spinosad formulation. The studies by Pine and Uner (2013, 2014) were conducted in Turkey. The Laser formulation used in these studies is produced by the Dow Chemical Company (http://msdssearch.dow.com/PublishedLiteratureDAS/dh_092e/0901b8038092e97b.pdf?filepath=it/pdfs/noreg/011-04057.pdf&fromPage=GetDoc) but does not appear to be marketed in the United States (i.e., the formulation is not listed at the CDMS website (https://www.cdms.net/), and U.S. labels for this formulation have not been identified. In any event, the concentration of 25 mg a.i./L—i.e., the lowest concentration assayed in the studies by Pine and Uner (2013, 2014—is more than 10 times higher than the NOAECs for fish on which the dose-response assessment is based (Section 4.3.3.1.1).

4.1.3.1.2. Longer-term Toxicity

Studies on the longer-term toxicity of spinosad to fish are summarized in Appendix 5, Table A5-2. Two of these studies are standard early life-stage studies submitted to the EPA and summarized in the most recent EPA ecological risk assessments (U.S. EPA/OPP/EFED 2005, 2009a, 2011a)—i.e., an assay in trout (MRID 43414541) and an assay in sheepshead minnow (MRID 44420601). A full DER is available for the assay in trout (Weinberg et al. 1993). In these studies, trout were somewhat more sensitive (NOAEC = 0.498 mg a.i./L) than sheepshead minnow (NOAEC = 1.15 mg a.i./L).

The review by Cleveland et al. (2002b) briefly summarizes a 21-day study in trout, reporting a NOAEC of 1.2 mg/L. A full citation for this this study is not given in the review, and this study is not cited in EPA risk assessments. The 21-day study in trout is simply designated as a …*21-day flow-through*. Because the *21-day flow-through* study reports a higher NOAEC than the early-life state study reviewed by EPA (MRID 43414541), the *21-day flow-through* study is cited for the sake of completeness but is not otherwise used in the current Forest Service risk assessment.

Two specialized studies in fish are published in the open literature (Anogwih et al. 2003; Elskus 2007). The study by Anogwih et al. (2002) is a micronucleus assay using mosquito fish exposure to spinosad (NOS) at concentrations of up to 0.361 mg/L. Micronucleus assays are sometimes used as mutagenicity screening tests. As discussed in Section 3.1.10, spinosad was assayed adequately for both carcinogenicity and mutagenicity, and these endpoints are not a concern—i.e., the assays for carcinogenicity and mutagenicity noted no positive activity. While Anogwih et al. (2003) note some statistically significant differences in nuclear morphology between control and treatment groups, the effects do not appear to be concentration dependent (Figure 1 of paper).

The study by Elskus (2007) is an assay for effects on immune function in zebra fish embryos. No responses suggestive of an effect on immune function were noted over the range of concentrations assayed (i.e., 0.2 to 30 ppb or µg/L). As discussed in Section 3.1.7, the lack of an immunotoxic response in fish is consistent with the determination in U.S. EPA/OPP/HED (2011a) that concern for the immunotoxicity of spinosad is low.

4.1.3.2. Amphibians (Aquatic Phase)

As with terrestrial phase amphibians, there are no data on the toxicity of spinosad to aquatic phase amphibians. The EPA ecological risk assessments on spinosad do not cite any registrant-submitted studies on aquatic-phase amphibians (i.e., U.S. EPA/OPP/EFED 2005, 2009a, 2010a, 2011a). The general lack of toxicity data on aquatic-phase amphibians extends to the open literature and the compendia of amphibian toxicity studies by Pauli et al. (2000). As noted in the EPA's most recent risk assessment on spinosad (U.S. EPA/OPP/EFED 2011a, p. 46), the EPA uses fish as a surrogate for aquatic-phase amphibians.

4.1.3.3. Aquatic Invertebrates

4.1.3.3.1. Acute Toxicity

Studies on the acute toxicity of spinosad to aquatic invertebrates are summarized in Appendix 5, Table A5-1. Acute toxicity values expressed in units of water concentration consist primarily of LC_{50} values (concentrations estimated to cause 50% mortality) and EC_{50} values (concentrations estimated to cause a non-lethal response in 50% of the organisms assayed) for aquatic invertebrates. An overview of the studies reporting acute LC_{50} or EC_{50} values is given in Table 25. For aquatic invertebrates, the distinction between LC_{50} and EC_{50} values is often unclear in publications, and the two terms may be used loosely and sometimes interchangeably. For very small invertebrates, EC_{50} values based on immobility can be readily determined while LC_{50} values (often based on lack of heart beat) may be difficult to determine. Cleveland et al. (2002b) as well as some studies on individual spinosyns (discussed further below) report both LC_{50} and EC_{50} values for *Daphnia magna*. As would be expected, the LC_{50} values are substantially higher than EC_{50} values. When both LC_{50} and EC_{50} values are available, the current discussion focusses on EC_{50} values. Functionally, immobility in a natural environment is equivalent to mortality. As discussed further in Section 4.3.3, the dose-response assessment for aquatic invertebrates is concerned primarily with estimated no effect levels; however, LC_{50} and EC_{50} values are generally preferable in estimating differences in sensitivity among species (e.g., Awkerman et al. 2008).

4.1.3.3.1.1. Daphnids

Daphnia magna is the most common freshwater invertebrate used in EPA risk assessments. Based on the EC_{50} of 14 mg a.i./L for spinosad (Milazzo et al. 1994, MRID 43574502), the EPA classifies spinosad as *slightly toxic* to freshwater invertebrates (U.S. EPA/OPP/EFED 2011a, p.31). As summarized in Table 5, some Material Safety Data Sheets give an LC_{50} of 1.5 mg a.i./L for spinosad in *Daphnia magna*. This toxicity value is not referenced in any of the EPA risk assessments on spinosad.

As summarized in Appendix 4, Table A5-1, toxicity studies in *Daphnia magna* were conducted on spinosyn A and spinosyn D—i.e., the components of spinosad—as well as several degradates of spinosad. Of the two components of spinosad, spinosyn A (the major component) is less toxic than spinosyn D (the minor component). Spinosyn A is classified as *Practically Nontoxic* based on an indefinite EC_{50} of >197 mg/L (MRID 46505307). Spinosyn D is classified as *Moderately Toxic* based on a definitive EC_{50} of 3.8 mg/L (MRID 46505307). Spinosyn B, a demethylated degradate of spinosyn A, is also somewhat more toxic than spinosad and is also classified as *Moderately Toxic* based on an EC_{50} values of 6.5 mg/L (MRID 46505312) and 6.49 mg/L (MRID 44597731).

While the data on *Daphnia magna* from registrant-submitted studies are relatively straightforward, the open literature on spinosad in daphnids and other invertebrates is more complex and (for some species) inconsistent. As summarized in Table 25, the lowest EC_{50} for spinosad is 0.0018 mg a.i./L based on a bioassay of Success® formulation in *Ceriodaphnia dubia* (Deardorff and Stark 2009). This study was conducted at Washington State University and was sponsored by the National Oceanic and Atmospheric Administration (NOAA). As summarized in Table 3, Success® is one of the representative formulations of spinosad covered in the current Forest Service risk assessment. Thus, at least for acute toxicity, the low EC_{50} in *Ceriodaphnia dubia* is relevant to the current risk assessment. As also summarized in Table 25 and detailed in Appendix 5, Table A5-1, Deardorff and Stark (2009) also conducted assays with the Success® formulation in *Daphnia magna* and *Daphnia pulex*. *Daphnia pulex* was the most tolerant of the three species of daphnids with an EC_{50} of 0.129 mg a.i./L. *Daphnia magna*, with an EC_{50} of 0.0048 mg a.i./L was more sensitive than *Daphnia pulex* but less sensitive than *Ceriodaphnia dubia*. Note that the EC_{50} for *Daphnia magna* from this study is lower than the EC_{50} of 14 mg/L from the registrant-submitted study used by EPA (MRID 43574502) by a factor of nearly 3000 [14 mg/L ÷ 0.0048 mg a.i./L ≈ 2916.66]. This substantial variability suggests that components in the Success® formulation other than spinosad contribute substantially to the toxicity of the formulation to *Daphnia magna*. As indicated in Table 3, one known inert ingredient in Success is propylene glycol (4% of the formulation). As summarized in HSDB (2015), propylene glycol has a very low toxicity to daphnids with an EC_{50} of >10,000 mg/L in *Daphnia magna* and 18,340 mg/L in a *Ceriodaphnia* species. Thus, it does not seem likely that propylene glycol could account for the higher toxicity of the Success® formulation relative to technical grade spinosad. Deardorff and Stark (2009) also examined the joint action of a surfactant (i.e., R-11) with the Success® formulation and note that the surfactant may enhance the toxicity of the formulation to *Ceriodaphnia dubia*. It is not known whether the Success® formulation contains a surfactant.

4.1.3.3.1.2. Mosquitoes

Some formulations of spinosad (e.g., Natular®) are labelled for aquatic applications to control mosquito larvae (e.g., Clarke 2011), and Table 25 summarizes the toxicity of spinosad to mosquito larvae. The maximum labelled rate for the control of mosquito larvae is 1.6 mg a.i./L (Jones and Ottea 2013).

Most of the available mosquito studies were conducted with *Culex quinquefasciatus* and are reasonably consistent with LC_{50} values generally ranging from 0.01 to 0.031 mg/L—i.e., a range that spans a factor of about 3. The LC_{50} of 0.1 mg a.i./L for technical grade spinosad is somewhat but not remarkably atypical. Jiang and Mulla (2009) matches studies on technical grade spinosad and an 11.6% a.i. formulation. The formulation is modestly more toxic than technical grade spinosad—i.e., an EC_{50} of 0.019 mg/L for technical grade versus an EC_{50} of 0.01 mg a.i./L for the formulation in 2nd instar larvae and an EC_{50} of 0.026 mg/L for technical grade versus an EC_{50} of 0.013 mg a.i./L for the formulation in 4th instar larvae. Similarly, Kovendan et al. (2012) note that earlier instar larvae are somewhat more sensitive than later instar larvae to spinosad—see Appendix 5, Table A5-1 for details. As discussed in Section 4.1.3.3.1.3, this pattern was observed also in midge larvae (Kumar et al. 2011). This is a typical pattern in aquatic toxicology with smaller organisms generally being more sensitive than larger

organisms, probably due to the greater surface area relative to body mass with decreasing body mass.

While the toxicity studies on *Culex quinquefasciatus* are reasonably consistent, the EC_{50} values for other species are less so. As summarized in Table 25, the reported EC_{50} values range by a factor of over 7000 for *Aedes aegypti* [51.7 mg/L ÷ 0.007 mg/L ≈ 7,386], a factor of about 16 for *Aedes albopictus* [0.3 mg/L ÷ 0.019 mg/L ≈ 15.79], a factor of 12 for *Anopheles stephensi* [0.024 mg/L ÷ 0.002 mg/L = 12], and a factor of about 27 for *Culex pipiens* [0.087 mg/L ÷ 0.0032 mg/L ≈ 27.18].

For *Aedes aegypti,* the very wide range in the toxicity values is due to the atypical EC_{50} of 51.7 mg a.i./L reported by Kovendan et al. (2012). This study was conducted using eggs collected from a field in India using a form of spinosad specified in the publication as material ... *obtained from T-Stanes & Company Limited, Research and Development Centre, Coimbatore, Tamil Nadu, India*. It is not clear if the spinosad was technical grade or a formulation. In addition, it is not clear if the population of *Aedes aegypti* was subject previously to substantial exposures to spinosad. As discussed further below, mosquitoes can develop resistance to spinosad but it does not seem likely that the high EC_{50} for *Aedes aegypti* would be due to resistance in a field population. Disregarding the study by Kovendan et al. (2012), the other three LC_{50} values for this species are consistent with the data on *Culex quinquefasciatus* as well as the data on *Daphnia magna* (4.1.3.3.1.1) indicating that the formulations are more toxic than technical grade spinosad. This pattern is to be expected for insecticide formulations used to control mosquito larvae, since it is reasonable to suppose that formulators would add other ingredients to the formulation to enhance the control of mosquito larvae.

The variability in toxicity values for *Aedes albopictus* is based on only two studies: a 24-hour EC_{50} of 0.3 mg a.i./L using technical grade spinosad (Liu et al. 2004b) and a 48-hour EC_{50} of 0.019 mg a.i./L using the Tracer® 24SC formulation (Khan et al. 2011). While this comparison is consistent with the greater toxicity of formulations relative to technical grade spinosad, the differences could also be due, at least partly, to the differences in exposure durations on which the EC_{50} values are based and differences in the sensitivity of the mosquito populations used in the studies.

As summarized in Table 24 and discussed in Section 4.1.2.4.6, resistance to spinosad is well documented in terrestrial insects with resistance factors of nearly 3 million in populations subject to artificial selection pressure in the laboratory and resistance factors of somewhat over 7000 in field populations. Resistance to spinosad in populations of mosquito larvae has also been demonstrated, although the number of studies in mosquito larvae is fewer than the number of studies in terrestrial insects. Under laboratory conditions with artificial selection pressure–i.e., 45 generations of *Culex quinquefasciatus* subject to spinosad concentrations equivalent to $LC_{70\text{-}90}$ values, resistance factors of somewhat over 1000 were noted (Su and Chen 2014b). In field populations not subject to artificial selection pressure, resistance factors of 23 to 50 were noted in populations of *Aedes albopictus* in Pakistan (Khan et al. 2011a), and resistance factors of 0.7 to 3 were noted in populations of *Culex quinquefasciatus* in Alabama (Jones and Ottea 2013; Liu et al. 2004a,b). Additional details of these studies are given in Appendix 5, Table A5-1.

Various factors can confound the assessment of species sensitivity differences in mosquito populations, including prior exposures of the insects to spinosad and/or other spinosyns, differences in the form of spinosad to which the mosquitos were exposed, and other experimental details. The study by Romi et al. (2006) is exceptional in that it provides matched bioassays on three species of mosquitoes. As summarized in Table 25, the order of sensitivities (most sensitive to least) is: Culex pipiens (EC_{50} = 0.0032 mg a.i./L), *Aedes aegypti* (EC_{50} = 0.007 mg a.i./L), and *Anopheles stephensi* (EC_{50} = 0.024 mg a.i./L). These bioassays were conducted with the same formulation (Laser®, 4.8% EC) using 3rd instar larvae from laboratory populations cultured for over 30 years.

4.1.3.3.1.3. Other Aquatic Invertebrates

In addition to the toxicity values for daphnids and mosquitoes, Table 25 summarizes acute toxicity values for midge larvae (*Chironomus circumdatus*), the eastern oyster (*Crassostrea virginica*), and grass shrimp (*Palaemonetes pugio*). Additional details for these studies are given in Appendix 6, Table A6-1. Based on the study by Kumar et al. (2011), midge larvae (EC_{50} = 0.009 mg/L) are nearly as sensitive as some daphnids and sensitive species of mosquitoes. The eastern oyster is much less sensitive with an EC_{50} of 0.3 mg/L (MRID 43571203). As indicated in the review by Cleveland et al. (2002b), the endpoint for the EC_{50} in oysters is new shell growth rather than immobility or mortality. The grass shrimp is highly tolerant with an LC_{50} of >9.67 mg/L (Cleveland et al. 2002b). Grass shrimp are much larger than daphnids, mosquitoes, and midge larvae, and the tolerance of the grass shrimp to spinosad is consistent with the general pattern of small aquatic invertebrates being more sensitive than larger aquatic invertebrates (Section 4.1.3.3.1.2). This study is cited in the U.S. EPA/OPP/EFED risk assessment on spinosad (2005, MRID 434145-39) and used to classify spinosad as *moderately toxic*.

In addition to the standard bioassays, other non-standard studies on the toxicity of spinosad to aquatic invertebrates are summarized at the end of Appendix 6, Table A6-1. Jones and Ottea (2013) examined mortality in three groups of nontarget aquatic invertebrates: damselflies *(Ischnura* sp., Odonata: Coenagrionidae), dragonflies, *(Pachydiplax* l*ongipennis*, Odonata: Libellulidae); and mayflies (*Caenis* sp., Ephemeroptera: Caenidae). The bioassays were conducted at two concentrations: 0.031 mg a.i./L (the LC_{50} for 3rd instar larvae of *Culex quinquefasciatus* as assayed by these investigators) and 1.6 mg a.i./L (the maximum application rate calculated by the investigators for a spinosad formulation used to control mosquito larvae). In these assays, mayflies were the most sensitive group with mortality in excess of 50% at both concentrations (Figure 2 of paper)—i.e., the mayflies appeared to be more sensitive than the mosquito species. Of the two Odonata species, damselflies were more sensitive than dragon flies; however, both species of Odonata appeared to be less sensitive than the mosquito species.

Infante-Rodriguez et al. (2011) examined the potential efficacy of spinosad for the control of black fly larvae (*Simulium* sp., Diptera: Simuliidae) in a series of short-term (10-minute pulse exposures) to black fly larvae as well as various groups of nontarget aquatic insect larvae. The 10-minute LC_{50} for black fly larvae was about 1.5 mg a.i./L. At a concentration of 12 mg a.i./L, a species of stonefly (*Anacroneura* sp., Plecoptera: Perlidae) was the only nontarget for which a significant increase in mortality was observed. A significant increase in mortality relative to

controls was not observed in mixed populations of Ephemeroptera (4 families, 5 species), Hemiptera (2 families, 2 species), Odonata (4 families, 4 species), and Trichoptera (3 families, 3 species).

4.1.3.3.2. Longer-term Toxicity

4.1.3.3.2.1. Spinosad

Information on the chronic toxicity of spinosad to aquatic invertebrates is summarized in Appendix 6, Table A6-2. An overview of these studies is given in Table 26. Note that units of µg/L rather than mg/L (used for acute studies) are used in Table 26 and in the following discussion because of the much lower toxicity values in chronic relative to acute exposures.

As discussed in Section 4.1.3.3.1 and summarized in Table 25, the available acute toxicity studies suggest that spinosad formulations may be more toxic than technical grade spinosad to daphnids. In addition, the study by Deardorff and Stark (2009) indicates that *Ceriodaphnia dubia* is substantially more sensitive than *Daphnia magna*. Neither of these patterns is apparent in the available chronic studies on daphnids. As summarized in Table 26, Deardorff and Stark (2011) conducted a reproduction study in *Ceriodaphnia dubia* using the same Success® formulation used in the acute toxicity studies. The chronic NOAEC of 0.5 µg a.i./L in *Ceriodaphnia dubia* is not substantially different from the chronic NOAEC of 0.62 mg a.i./L in *Daphnia magna* reported in U.S. EPA/OPP/EFED (2011a, MRID 43848801).

The European Commission (2006) reports somewhat higher NOAEC values in *Daphnia magna* based on a flow-through assay (NOAEC = 1.2 µg/L) and a static renewal assay (NOAEC = 8 µg a.i./L). The static renewal 21-day NOAEC of 8 µg/L from the European Commission (2006) is not consistent with the static renewal 14-day LOAEC of 8 µg/L in *Daphnia magna* from the study by Duchet et al. (2010b). As summarized in Appendix 6, Table A6-2, the review by Cleveland et al. (2002b, Table 3 of paper) reports an NOEC of 6.88 µg a.i./L for a …*21-day flow through 5-day pulsed*… exposure in *Daphnia magna*. Details of this study are not discussed in the review by Cleveland et al. (2002b), and this study is not summarized in the EPA ecological risk assessments.

In a somewhat unusual static renewal study, Stark and Vargas (2003) conducted a long-term population study in *Daphnia pulex* at concentrations ranging from 2 to 11 µg a.i./L. At concentrations of 10 and 11 µg a.i./L, all organisms died by about Day 10. At lower concentrations, populations survived for up to about 70 days (see Figure 1 of paper). A decrease in net reproductive rate (i.e., the number of offspring per generation) was observed at all concentrations (Figure 3 of paper). The LOAECs in *Daphnia pulex* reported by Stark and Vargas (2003) is supported by a LOAEC of 8 µg a.i./L in *Daphnia pulex* (Duchet et al. 2010b). Due to the lack of NOAELs from these studies, the sensitivity of *Daphnia pulex* relative to *Daphnia magna* and *Ceriodaphnia dubia* cannot be assessed. In a subsquent publication, Stark (2005) exposed a population (n=300) of *Daphnia pulex* to spinosad (source not specified) at a concentration of 129 µg a.i./L. As summarized in Table 25, 129 µg a.i./L is the 48 hour-LC_{50} for *Daphnia pulex* from the study by Deardorff and Stark (2009). As might be expected, none of the *Daphnia pulex* survived the 10 day exposure to 129 µg a.i./L (Stark S2005).

Table 25, also summarizes three chronic NOAECs for midge larvae. The lowest NOAEC is 0.622 µg a.i./L reported in U.S. EPA/OPP/EFED (2011a, MRID 44828402). This NOAEC is

virtually identical to the NOAEC of 0.62 µg a.i. for *Daphnia magna* also reported in U.S. EPA/OPP/EFED (2011a, MRID 43848801). Notably, the endpoint for chronic studies in midge larvae involves adult emergence rather than reproduction. Both the European Commission (2006) and Cleveland et al. (2002a) review report a chronic NOAEC in midge larvae of 1.6 µg a.i./L. It seems likely that these identical NOAECs reflect a single study; however, that cannot be determined clearly from the references cited in the European Commission (2006) and Cleveland et al. (2002a).

While the NOAECs for *Daphnia magna* and midge larvae are similar, mysid shrimp (*Mysidopsis bahia*) are much more tolerant, based on the reported chronic NOAEC of 84.2 µg a.i./L (U.S. EPA/OPP/EFED 2011a, MRID 47702901).

4.1.3.3.2.2. Components and Metabolites

The longer-term toxicity data on the components of spinosad (i.e., spinosyn A and spinosyn D) as well as the degradates of spinosad are summarized in Appendix 6, Table A6-2.

Based on NOAEC of 0.62 µg a.i./L in *Daphnia magna* (MRID 43848801), none of the metabolites is more toxic than spinosad. The β-13,14-dihydropseudo-aglycone degradation products of both spinosyn A (NOAEC = 4850 µg/L, MRID 46505303) and spinosyn D (NOAEC = 1590 µg/L, MRID 46505305) are much more toxic than technical grade spinosad. Unlike the case with acute toxicity in *Daphnia magna*, Spinosyn B, a demethylated degradate of spinosyn A, as well as a demethylated degradate of spinosyn D are modestly less toxic than spinosad—i.e., an NOAEC for spinosyn B of 0.95 µg/L and a NOAEC of 1 µg/L for N-demethylated spinosyn D (European Commission 2006).

As with *Daphnia magna*, none of the data on the degradates of spinosad are shown to be more toxic to midge larvae than spinosad itself based on NOAEC of 0.622 µg a.i./L in midge larvae (MRID 44828402). On the other hand, there are two equivocal studies that report indefinite NOAECs (i.e., studies in which LOAECs were not defined). These studies include an assay of N-demethylated spinosyn D which yielded an indefinite NOAEC of 0.14 µg a.i./L (MRID 46505315) and an assay of N-demethylated spinosyn A which yielded an indefinite NOAEC of 0.41 µg a.i./L (MRID 46505315). All other studies on midge larvae using degradates of spinosad yielded NOAECs higher than spinosad itself.

Also, as with acute toxicity in *Daphnia magna* (Section 4.1.3.3.1), one chronic study in midge larvae indicates that spinosyn A, the major component in spinosad, is much less toxic than technical grade spinosad—i.e., the chronic indefinite NOAEC in midge larvae of 73.4 µg a.i./L (MRID 46505314).

4.1.3.3.3. Microcosm/Mesocosm Studies

The microcosm and mesocosm studies concerning the effects of spinosad on aquatic invertebrates are summarized in Appendix 6, Table A6-3. The studies include effects on daphnids [Cladocera: Daphniidae] (Duchet et al. 2008; Duchet et al. 2010a), midges [Diptera: Chironomidae] (Duchet et al. 2015), mosquitoes [Diptera: Culicidae] (Lawler and Dritz 2013; Jiang and Mulla 2009), and mayflies [Ephemeroptera: Baetidae] (Lawler and Dritz 2013). For the sake of clarity, it is noted that Duchet et al. (2008, 2010a, 2015) and Jiang and Mulla (2009) refer to their studies as microcosms. These as well as the other studies summarized in Appendix

6, Table A6-3, involve outdoor and relatively complex systems that could be referred as mesocosms rather than outdoor microcosms (e.g., Suter and Bartell 1993).

None of the mesocosm studies notes effects that are inconsistent with the more controlled chronic studies discussed in Section 4.1.3.3.2. In the 21-day mesocosm study on *Daphnia pulex*, Duchet et al. (2008) note that adverse effects (i.e., decreased body length) occurred at concentrations as low as 2 µg/L and view this as …*inconsistent with the laboratory data published by Stark (2005), who estimated the acute LC_{50} at 129 µg/L* (Duchet et al. 2008, p. 76). As summarized in Table 26, the study by Stark (2005) involves a 10-day exposure of *Daphnia pulex* to 129 µg a.i./L, a concentration equivalent to the 48-hour LC_{50} from the study by Deardorff and Stark (2009). Nonetheless, the adverse effects on *Daphnia pulex* in the mesocosm study by Duchet et al. (2008) at concentrations as low as 2 µg a.i./L are consistent with the reproductive LOAEC of 2 µg a.i./L from the chronic study in *Daphnia pulex* (i.e., Stark and Vargas 2003 as summarized in Table 26).

In a subsequent mesocosm study in *Daphnia magna*, Duchet et al. (2010a) observed decreases in daphnid abundance at concentrations as low as 2 µg a.i./L over a 21-day period of exposure. As summarized in Table 26, this observation is consistent with the LOAEC of 1.2 µg a.i./L and a corresponding NOAEC of 0.62 µg a.i./L in *Daphnia magna* from MRID 43848801 as summarized in U.S. EPA/OPP/ EFED (2011a) and Cleveland et al. (2002b). More recently, Duchet et al. (2015) conducted a similar mesocosm study on two species of midge larvae [Diptera: Chironomidae] and observed adverse effects (decreased emergence) at concentrations of 8 µg a.i./L in *Polypedilum nubifer* and at 17 µg a.i./L in *Tanytarsus curticornis*. These species were not assayed in standard chronic laboratory studies; nonetheless, the LOAECs from the mesocosm study are substantially above the LOAEC of 1.328 µg a.i./L from the standard chronic bioassay in *Chironomus riparius* (MRID 44828402 as summarized in Table 26 and detailed in Appendix 6, Table A6-2).

Jiang and Mulla (2009) is a relatively standard efficacy study in mosquitoes, *Culex quinquefasciatus*. The satisfactory control of mosquito larvae at concentrations of 50 µg a.i./L and higher is consistent with the 48 hour-LC_{50} of about 0.01 mg a.i./L in this species (Table 25). The study by Lawler and Dritz (2013) expresses exposures in terms of application rates in lb/acre rather than concentrations of spinosad in water. This study is interesting, however, in noting that nontarget Ephemeroptera (i.e., *Callibaetis californicus* nymphs) are more sensitive than larvae of the target mosquito species, *Culex tarsalis* [Diptera: Culicidae] and chironomid midge larvae. The lesser sensitivity of Ephemeroptera relative to Diptera is noted in short-term studies by Infante-Rodriguez et al. (2011); however, the opposite pattern is noted in acute toxicity studies conducted by Jones and Ottea (2013) (Section 4.1.3.3.1.3).

4.1.3.4. Aquatic Plants

4.1.3.4.1. Algae

Information on the toxicity of spinosad and its metabolites is summarized in Appendix 7, Table A7-1. All of the information on algae is taken from reviews or EPA risk assessments (Cleveland et al. 2002b; U.S. EPA/OPP /EFED 2011a; European Commission 2006). The open literature does not appear to include studies on the toxicity of spinosad to algae.

The most sensitive species is the freshwater diatom, *Navicula pelliculosa*, with EC_{50} values for technical grade spinosad ranging from 0.09 mg a.i./L (MRID 43414543) to 0.135 mg a.i./L (Cleveland et al. 2002b). Notably, both of these studies report a NOAEC of 0.05 mg a.i./L; thus, it seems reasonable to conclude that these are summaries of the same study (conducted by or for Dow AgroSciences and then submitted to EPA) and that the relatively minor difference in the EC_{50} values reflect a reanalysis of the dose-response data by EPA. Based on the 5-day EC_{50} values reported in the European Commission (2006) review for technical grade spinosad (EC_{50} = 0.079 mg a.i./L) and the NAF-85 formulation (EC_{50} = 0.35 mg a.i./L), the other ingredients in the formulation do not appear to contribute to and may, in fact, reduce its toxicity to this species of algae. While the European Commission (2006) does not describe the formulation other than using the NAF-85 designation, this designation is identified in EPA/OPP/HED (1997b) as the Tracer® formulation (44.2% a.i.).

As also summarized in Appendix 7, Table A7-1, *Navicula pelliculosa* has been used in bioassays of several degradates of spinosad. Based on definitive EC_{50} values, most of the degradates appear to be much less toxic than spinosad with EC_{50} values ranging from 0.16 mg a.i./L (N-demethyl-A) to 38.8 mg a.i./L (the β-13,14-dihydropseudo-aglycone degradate of spinosyn A). The only exception involves the toxicity of spinosyn B, demethylated degradate of spinosyn A. Based on the EC_{50} values given in the European Commission (2006) review, the spinosyn A (EC_{50} = 0.077 mg a.i./L) is about equitoxic to technical grade spinosad (0.079 mg a.i./L). This is similar to the pattern in *Daphnia magna* in which spinosyn B was somewhat more toxic than technical grade spinosad (Section 4.1.3.3.1.1).

Other species of algae are much less sensitive to spinosad with EC_{50} values ranging from 0.227 mg a.i./L (*Skeletonema costatum*) to >105.5 mg a.i./L (*Selenastrum capricornutum*) with both of these EC_{50} values reported in Cleveland et al. (2002b). The minimal toxicity of spinosad to *Selenastrum capricornutum* is supported by an indefinite EC_{50} of >48 mg a.i. for the NAF-85 formulation (European Commission 2006).

4.1.3.4.2. Aquatic Macrophytes

The only information on the toxicity of spinosad to aquatic macrophytes is a standard 7-day study in duckweed, *Lemna gibba* (MRID 43414546), that yielded an EC_{50} for growth of 10.6 mg a.i./L with a corresponding NOAEC of 1.86 mg a.i./L. This study is briefly summarized in U.S. EPA/OPP/EFED (2011a) and Cleveland et al. (2002b). The open literature does not appear to include other information on the toxicity of spinosad to aquatic macrophytes

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview

A standard set of exposure assessments for terrestrial and aquatic organisms is provided in the EXCEL workbooks for spinosad. Attachment 1 details the exposure assessments for foliar applications at the anticipated application rate of 0.225 lb a.i./acre. Attachment 2 covers two applications at the rate of 0.225 lb a.i./acre with a 6-day application interval. As with the exposure assessment for human heath (Section 3.2), all exposure assessments involving applications of spinosad are expressed in units of active ingredient (a.i.).

As in the human health risk assessment, three general types of exposure scenarios are considered: accidental, acute non-accidental, and longer-term. Exposure assessments are detailed in Worksheet G01a for mammals and in Worksheet G01b for birds. For both mammals and birds, the highest exposure scenarios are associated with the consumption of contaminated vegetation. This is a common pattern for applications of any pesticide to vegetation. The highest exposures are associated with the consumption of contaminated short grass by a small mammal or bird.

Exposure scenarios for honeybees and phytophagous insects are also considered quantitatively. Forest Service risk assessments of insecticides typically assess risks to honeybees based on a direct spray scenario and pathways for direct spray and spray drift are considered. For phytophagous insects and foraging honeybees, exposures are estimated, although the information used to estimate exposures is based on different data sets for the two groups of terrestrial invertebrates.

Exposures of aquatic animals and plants are based on essentially the same information used to assess the exposure to terrestrial species from contaminated water (Section 3.2.3.4.6).

4.2.2. Mammals and Birds

All of the exposure scenarios that are more or less standard in Forest Service risk assessments for broadcast applications are not relevant to the foliar and bark application methods considered in the current risk assessment of spinosad.

Table 27 provides an overview of the mammalian and avian receptors considered in the current risk assessment. These data are discussed in the following subsections. Because of the relationship of body weight to surface area as well as to the consumption of food and water, the dose for smaller animals is generally higher, in terms of mg/kg body weight, than the dose for larger animals. Consequently, the exposure assessment for mammals considers five nontarget mammals of varying sizes: small (20 g) and medium (400 g) sized omnivores, a 5 kg canid, a 70 kg herbivore, and a 70 kg carnivore. Four standard avian receptors are considered: a 10 g passerine, a 640 g predatory bird, a 2.4 kg piscivorous bird, and a 4 kg herbivorous bird. Because of presumed differences in diet, (i.e., the consumption of food items), all of the mammalian and avian receptors are not considered in all of the exposure scenarios (e.g., the 640 g predatory bird is not used in the exposure assessments for contaminated vegetation).

4.2.2.1. Direct Spray

Direct spray scenarios are relevant to the foliar applications of virtually any pesticide. In a scenario involving exposure to direct spray, the amount of pesticide absorbed depends on the application rate, the surface area of the organism, and the rate of absorption. For this risk assessment, two direct spray or broadcast exposure assessments are conducted. The first spray scenario (Worksheet F01a) concerns the direct spray of half of the body surface of a 20 g mammal during a pesticide application. This exposure assessment assumes first-order dermal absorption using the first-order dermal absorption rate coefficient (k_a) discussed in Section 3.1.3.2.2. The second exposure assessment (Worksheet F01b) assumes complete absorption over Day 1 of exposure. This assessment is included in an effort to encompass increased exposures due to grooming.

Exposure assessments for the direct spray of a large mammal are not developed. As discussed further in Section 4.4.2.1, the direct spray scenarios lead to HQs far below the level of concern, and an elaboration for body size would have no impact on the risk assessment.

4.2.2.2. Dermal Contact with Contaminated Vegetation

As discussed in the human health risk assessment (Section 3.2.3.3), the approach for estimating the potential significance of dermal contact with contaminated vegetation is to assume a relationship between the application rate and dislodgeable foliar residue as well as a transfer rate from the contaminated vegetation to the skin. Unlike the human health risk assessment for which estimates of transfer rates are available, there are no transfer rates available for wildlife species. Wildlife species are more likely than humans to spend long periods of time in contact with contaminated vegetation. It is reasonable to assume that for prolonged exposures, equilibrium may be reached between pesticide levels on the skin, rates of dermal absorption, and pesticide levels on contaminated vegetation. The lack of data regarding the kinetics of this process precludes a quantitative assessment for this exposure scenario.

For spinosad, the failure to quantify exposures associated with dermal contact adds relatively little uncertainty to the risk assessment, since the consumption of contaminated vegetation is the greatest source of exposure, as discussed below (Section 4.2.2.3).

4.2.2.3. Ingestion of Contaminated Vegetation or Prey

The exposure scenarios for the consumption of contaminated vegetation are similar to the exposure scenarios considered in the human health risk assessment (Section 3.2.3.7), except that the ecological risk assessment considers a wider variety of vegetation—i.e., long and short grass, in addition to fruit and broadleaf vegetation, which are considered in the human health risk assessment.

The acute and chronic exposure scenarios are based on the assumption that 100% of the diet is contaminated, which may not be realistic for some acute exposures and seems an unlikely event in chronic exposures to birds or larger mammals which may move in and out of the treated areas over a prolonged period of time. While estimates of the proportion of the diet contaminated could be incorporated into the exposure assessment, the estimates would be an essentially arbitrary set of adjustments. The proportion of the contaminated diet is linearly related to the resulting HQs, and its impact is discussed further in the risk characterization (Section 4.4.2).

As summarized in Table 27, the estimated food consumption rates by various species of mammals and birds are based on field metabolic rates (kcal/day), which, in turn, are based on the adaptation by the U.S. EPA/ORD (1993) of estimates from Nagy (1987). These allometric relationships account for much of the variability in food consumption among mammals and birds. There is, however, residual variability, which is remarkably constant among different groups of organisms (Table 3 in Nagy 1987). As discussed by Nagy (2005), the estimates from the allometric relationships may differ from actual field metabolic rates by about ±70%. Consequently, in all worksheets involving the use of the allometric equations for field metabolic rates, the lower bound is taken as 30% of the estimate and the upper bound is taken as 170% of the estimate.

The estimates of field metabolic rates are used to calculate food consumption based on the caloric value (kcal/day dry weight) of the food items considered in this risk assessment and estimates of the water content of the various foods. Estimates of caloric content are summarized in Table 28. Most of the specific values in Table 28 are taken from Nagy (1987) and U.S. EPA/ORD (1993).

Along with the exposure scenarios for the consumption of contaminated vegetation, similar sets of exposure scenarios are provided for the consumption of small mammals by either a predatory mammal (Worksheet F10a) or a predatory bird (Worksheet F10b) and the consumption of contaminated insects by a small mammal, a larger (400 g) mammal, and a small bird (Worksheets F09a-c).

4.2.2.4. Ingestion of Contaminated Water

The methods for estimating spinosad concentrations in water are identical to those used in the human health risk assessment (Section 3.2.3.4) using the water contamination rates (mg a.i./L of water per lb a.i./acre), as summarized in Table 14. In the attachments to this risk assessment, the water contamination rates are entered into Worksheet B04Rt and adjusted to expected concentrations in water in Worksheet B04a.

Body weight and water consumption rates are the major differences in the exposure estimates for birds and mammals, relative to humans. Like food consumption rates, water consumption rates, which are well characterized in terrestrial vertebrates, are based on allometric relationships in mammals and birds, as summarized in Table 27.

Like food consumption, water consumption in birds and mammals varies substantially with diet, season, and many other factors. Quantitative estimates regarding the variability of water consumption by birds and mammals are not well documented in the available literature and are not considered in the exposure assessments. As discussed further in Section 4.4.2.1 (risk characterization for mammals) and Section 4.4.2.2 (risk characterization for birds), exposures associated with the consumption of contaminated surface water are far below the level of concern (HQ=1). Consequently, extreme variations in the estimated consumption of contaminated water by mammals and birds would have no impact on the risk characterization for mammals and birds.

4.2.2.5. Consumption of Contaminated Fish

In addition to the consumption of contaminated vegetation, insects, and other terrestrial prey (Section 4.2.2.3), the consumption of contaminated fish by piscivorous species is a potentially significant route of exposure to spinosad. Exposure scenarios are developed for the consumption of contaminated fish after an accidental spill (Worksheets F03a-c), expected peak exposures (Worksheets F011a-c), and estimated longer-term concentrations (Worksheets F17a-c). These exposure scenarios are applied to 5 and 70 kg carnivorous mammals as well as a 2.4 kg piscivorous bird. The 70 kg carnivorous mammal is representative of a small or immature brown bear (*Ursus arctos*), which is an endangered species that actively feeds on fish (Reid 2006). As summarized in Table 27, the 5 kg mammal is representative of a fox, and the 2.4 kg bird is representative of a heron.

Spinosad exposure levels associated with the consumption of contaminated fish depend on the spinosad concentration in water and the bioconcentration factor for spinosad in fish. The concentrations of spinosad in water are identical to those discussed in Section 4.2.2.4. The bioconcentration factor for whole fish is taken as 84 for acute exposures and a BCF of 115 for longer-term exposures. As summarized in Table 2, these BCF values are within the range of BCFs summarized in U.S. EPA/OPP/EFED (2009a, p. 8). Given the relationship between exposure time and bioconcentration, the lower bound of 84 is used for acute exposures and the upper bound of 115 is used for longer-term exposures.

4.2.3. Terrestrial Invertebrates

4.2.3.1. Direct Spray and Drift

Estimated levels of exposure associated with broadcast terrestrial applications of spinosad are detailed in Worksheet G09 of Attachments 1 and 2 (the EXCEL workbooks for spinosad). In these attachments, Worksheet G09 is a custom worksheet which includes aerial, ground broadcast (high boom and low boom), and backpack applications.

Honeybees are used as a surrogate for other terrestrial insects, and honeybee exposure levels associated with broadcast applications are modeled as a simple physical process based on the application rate and planar surface area of the bee. The planar surface area of the honeybee (1.42 cm^2) is based on the algorithms suggested by Humphrey and Dykes (2008) for a bee with a body length of 1.44 cm.

The amount of a pesticide deposited on a bee during or shortly after application depends on how close the bee is to the application site as well as foliar interception of the spray prior to deposition on the bee. The estimated proportions of the nominal application rate at various distances downwind given in G09 are based on Tier 1 estimates from AgDRIFT (Teske et al. 2002) for distances of 0 (direct spray) to 900 feet downwind of the treated site. Further details of the use of AgDRIFT are discussed in SERA (2014a, 4.2.4.2) and SERA (2011b, Section 3.3.2).

In addition to drift, foliar interception of a pesticide may occur. The impact of foliar interception varies according to the nature of the canopy above the bee. For example, in studies investigating the deposition rate of diflubenzuron in various forest canopies, Wimmer et al. (1993) report that deposition in the lower canopy, relative to the upper canopy, generally ranged from about 10%

(90% foliar interception in the upper canopy) to 90% (10% foliar inception by the upper canopy). In Worksheet G09, foliar interception rates of 0% (no interception), 50%, and 90% are used.

During broadcast applications of a pesticide, it is likely that terrestrial invertebrates other than bees will be subject to direct spray. As discussed in Section 4.1.2.4.1, summarized in Table 19, and detailed further in Section 4.3.2.3 (dose-response assessment for terrestrial invertebrates), toxicity data on other terrestrial invertebrates suggest that honeybees are the most sensitive species of terrestrial invertebrates for which contact toxicity data are available.

4.2.3.2. Ingestion of Contaminated Vegetation or Prey

Like terrestrial mammals and birds, terrestrial invertebrates may be exposed to spinosad through the consumption of contaminated vegetation or contaminated prey. As with consumption scenarios for humans (Section 3.2.3.7) and mammalian wildlife (Section 4.2.3.2), estimates of residues on contaminated vegetation or prey are based on estimated residue rates (i.e., mg/kg residues per lb applied) from Fletcher et al. (1994), as summarized in Table 15.

An estimate of food consumption is necessary to calculate a dose level for a foraging herbivorous insect. Insect food consumption varies greatly, depending on the caloric requirements in a given life stage or activity of the insect and the caloric value of the food to be consumed. The derivation of consumption values for specific species, life stages, activities, and food items is beyond the scope of the current analysis. Nevertheless, general food consumption values, based on estimated food consumption per unit body weight, are readily available.

Reichle et al. (1973) studied the food consumption patterns of insect herbivores in a forest canopy and estimated that insect herbivores may consume vegetation at a rate of about 0.6 of their body weight per day (Reichle et al. 1973, pp. 1082 to 1083). Higher values (i.e., 1.28-2.22 in terms of fresh weight) are provided by Waldbauer (1968) for the consumption of various types of vegetation by the tobacco hornworm (Waldbauer 1968, Table II, p. 247). The current risk assessment uses food consumption factors of 1.3 (0.6 to 2.2) kg food /kg bw. The lower bound of 0.6 is taken from Reichle et al. (1973), and the central estimate and upper bound are taken from the range of values provided by Waldbauer (1968).

A summary of the estimated exposures in terrestrial herbivorous insects is given in Worksheet G08a, and details of the calculations for these scenarios are provided in Worksheets G07a, G07b, G07c, and G07d of the EXCEL workbooks that accompany this risk assessment (Attachments 1 and 2). These levels pertain to the four food items included in the standard residue rates provided by Fletcher et al. (1994), as summarized in Table 15.

4.2.3.3. Nectar Exposures Involving Honeybees

4.2.3.3.1 General Method

Prompted by concerns raised in a Tier 1 analysis for imidacloprid conducted by the Forest Service (Appleton 2008), the basic approach taken in the current risk assessment as well as an earlier Forest Service risk assessment on dinotefuran (SERA 2009) is conceptually similar to the analysis of the potential impact of imidacloprid on honeybees developed for the French Ministry of Agriculture (Alix and Vergnet 2007; Halm et al. 2006; Rortais et al. 2005). The analyses conducted for the French Ministry of Agriculture develop imidacloprid exposure assessments for

several subgroups of honeybees (i.e., nectar foragers, pollen foragers, larvae, brood attending bees, and winter bees). As in the risk assessment for dinotefuran (SERA 2009), the current risk assessment for spinosad is limited only to nectar foragers because this is the subgroup estimated to be exposed to the highest dose (Rortais et al. 2005, p. 73, Table 1). Analogous to the approach taken in the human health risk assessment (Section 3.2.3.1.1), a nectar forager is taken as the Most Exposed Individual (MEI).

The basic algorithm for estimating the daily dose (D) to the foraging bee, based on the nutritional requirements of the bee is:

$$D_{mg/kg\ BW} = C_{Nec\,mg/L} \times Am_{Nec_L} \div BW_{kg} \quad (7)$$

where:

C = Concentration of pesticide in nectar in units of mg/L
Am = Amount of nectar in liters consumed by a foraging bee per day based on the nutritional requirements of the bee.
BW = Body weight of the bee in kilograms.

The amount of nectar a bee needs to consume is calculated from the nutritional requirements of the bee. Nutritional requirements for bees are generally expressed in the literature as the amount of sugar per unit time. Rortais et al. (2005) express the sugar requirement of bee during flight as 8 - 12 mg/hour, which is reasonably close to the value of 11.5 mg/hour cited by Winston (1987). The current risk assessment uses a sugar requirement for flight of 10 (8 - 12) mg/hour.

The number of hours/day that a bee might spend foraging is likely to be highly variable. Rortais et al. (2005) use a range from 4 to 10.7 hours/day. This range is used in the current exposure assessment on spinosad with a central estimate of 6.5 hours/day, the approximate geometric mean of the lower and upper bounds from Rortais et al. (2005).

Thus, the amount(s) of sugar ($Am_{SugarFl}$) required by a bee to support flight activities during foraging is calculated as the product of the sugar requirements per hour during flight and the number of hours/day that the bee spends in flight:

$$Am_{Sugar\ FL} = Rate_{mg/h} \times Fight_{h/day}$$
$$Am_{Sugar\ FL} = 10\,(8\,to\,12)_{mg/h} \times 6.5\,(4\,to\,10.7)_{h/day} \quad (8)$$

Using the above equation, the amount(s) of sugar required per day to support flight activities is calculated as 65.5 (32 - 128.4) mg/day.

Rortais et al. (2005) base their exposure assessment only on sugar requirements during flight. In the current Forest Service risk assessment of spinosad, the estimated nutritional requirement also includes time at rest, using the value of 0.7 mg/hour from Winston (1987, p. 61). From the same equation used above, the sugar requirement(s) for hours other than those engaged in flight is calculated as:

$$Am_{Sugar\ Oth} = 7_{mg/h} \times 24_{h/day} - 6.5\,(4\,to\,10.7)_{h/day} \quad (9)$$

which is equivalent to 12.25 (14 to 9.31) mg/day.

Thus, the total sugar requirement(s) per day for a foraging honeybee is calculated as:

$$Am_{Sugar\ Total} = Am_{Sugar\ Flt} + Am_{Sugar\ Oth}$$
$$Am_{Sugar\ Total} = 65\,(32\ to\ 128.4)_{mg/day} + 12.25\,(14\ to\ 9.31)_{mg/day} \quad (10)$$

which is equivalent to 77.25 (46 to 137.71) mg/day. Compared with the method used by Rortais et al. (2005), the inclusion of metabolic requirements during non-flight hours increases the sugar demand by about 20%.

The sugar content of nectar also varies among plants and locations. Rortais et al. (2005) uses a value of 0.4—i.e., nectar consists of 40% w/w nutritional sugars. This single value is also used in the current risk assessment. So, when the sugar requirement(s) is divided by 0.4 (mg sugar/mg nectar), the estimated amount of nectar required per day is about 193 (115 - 344) mg/day. In the worksheets for this exposure scenario (i.e., G10 in the attachments), these values are converted to units of kg nectar per day by dividing mg/day by 1,000,000 mg/kg.

The exposure assessments in the EXCEL workbooks are based on honey and not nectar consumption. This approach is inconsequential since the basis of the exposure assessment is the energy requirement of the bee. As discussed by Rortais et al. (2005, p. 73, column 2),

> *As we do not know the bees' differential consumption of nectar and honey, we related their sugar consumption depending on whether they consume nectar or honey. With the example of sunflower, when a honeybee requires 1 mg of sugar, it will have to consume either 2.5 mg of fresh sunflower nectar or 1.25 mg of sunflower honey.*
>
> – Rortais et al. 2005, p. 73

In other words, the amount of spinosad consumed by the bee would be the same whether the exposure is based on nectar consumption or honey consumption.

Another uncertainty in the amount of contaminated nectar that a foraging honeybee might consume involves the proportion of the plants that are contaminated in the area in which the honeybee forages. For broadcast applications, this factor is inconsequential as it seems reasonable to assume that 100% of the plants would be contaminated. More focused application methods, such as directed foliar, could and probably would generally result in a highly uneven distribution of spinosad over the general area in which the applications occur. Nonetheless, the assumption used in the current risk assessment is that backpack applications would be done at the nominal application rate of 0.225 lb a.i./acre which could be viewed as a functional average over the treated area.

4.2.3.3.2. Concentrations of Spinosad in Nectar

Data on the concentration of spinosad in nectar following its application at a known application rate are not addressed in the available literature. Following a foliar application of a Success®

480SC formulation at an application rate of 40 g a.i./ha (≈0.0357 lb a.i./acre), Bailey et al. (2005) detected spinosad in sweet corn pollen at a concentration of 0.32 mg a.i./kg pollen (Bailey et al. 2005, p. 630, Table IV). In the absence of additional relevant information, a contamination rate for pollen is calculated as about 8.96 mg a.i./kg pollen per lb a.i./acre [0.32 mg a.i./kg pollen ÷ 0.0357 lb a.i./acre ≈ 8.9636 mg a.i./kg pollen per lb a.i./acre].

The contamination rate for pollen is used to estimate a contamination rate for nectar using the study by Dively and Kamel (2012). These investigators monitored concentrations of three insecticides (i.e., imidacloprid, dinotefuran, and thiamethoxam) in both pollen and nectar of flowering pumpkin plants (*Cucurbita pepo*). As summarized in Table 29, the ratios of nectar to pollen covered a relatively narrow range (i.e., 0.08 to 0.16) with a mean and 95% confidence interval of 0.12 (0.099 – 0.15). This mean and confidence interval on the ratios of nectar-to-pollen are multiplied by the 8.96 mg a.i./kg pollen per lb a.i./acre to estimate a contamination rate for nectar of 1.08 (0.89 – 1.34) mg a.i./kg nectar per lb a.i./acre.

The lack of field monitoring data on the concentrations of spinosad in the nectar of wild flowers that might be foraged by bees following Forest Service applications of spinosad is an obvious and substantial limitation, as discussed further in the risk characterization for pollinators (Section 4.4.2.4.3).

4.2.3.3.3. Exposure Estimates

Details of the exposure scenario for foraging honeybees are given in Worksheet G10 of the attachments to this risk assessment based on the method detailed in Section 4.2.3.3.1, and the estimated concentration of spinosad in nectar is detailed in Section 4.2.3.3.2. The implementation of the exposure assessment for a single application (Attachment 1) is straightforward. For two applications (Attachment 2), an estimate of the half-life of spinosad in nectar is required. In the absence of additional information, the half-lives used for this exposure assessment are identical to the half-lives used in the GLEAMS-Driver modeling – i.e., 6 (1.5 – 35) days. As with the estimates of the concentration of spinosad in nectar (Section 4.2.3.3.2), the lack of data on the kinetics of spinosad in nectar is a substantial uncertainty also addressed in the risk characterization for pollinators (Section 4.4.2.4.3).

4.2.3.4. Concentrations in Soil

As discussed in Section 4.1.2.4.7, toxicity data are available on earthworms. The GLEAMS modeling discussed in Section 3.2.3.4 provides estimates of soil concentration as well as estimates of off-site movement (runoff, sediment, and percolation). Based on the GLEAMS modeling conducted at a unit application rate of 1 lb a.i./acre, spinosad concentrations in clay, loam, and sand soil textures over a broad range of rainfall rates are summarized in Appendix 8 for a single application and Appendix 9 for two applications with a 6-day application interval. Table 2 in each of these appendices gives the estimated concentration of spinosad in the top 12 inches of the soil column at a normalized application rate of 1 lb/acre. Table 3 in these appendices gives the corresponding values for the top 36 inches of soil. Analogous to the approach taken with water contamination rates (Table 14), a summary of the modeled soil concentrations is presented in Table 30. Note that the soil concentration rates in this table are given in units of mg spinosad/kg soil (ppm) per lb a.i./acre. As indicated in Appendices 8 and 9, the concentrations for clay soil textures are somewhat higher than those for loam and sand. Thus, only the estimates for clay soil textures are given in Table 30. As discussed further in

Section 4.4.2.4.4, these concentration rates lead to exposure estimates far below levels that would be of concern for earthworms.

4.2.3.5. Contact with Contaminated Surfaces

As in the human health risk assessment (Section 3.2.3.3) and vertebrate wildlife (Section 4.2.2.2), the contact of terrestrial invertebrates with contaminated vegetation is a potential exposure route of concern. Insects are likely to come into contact with spinosad on contaminated surfaces after directed or broadcast applications; however, data and methods to quantify this type of exposure in terms of mg/kg bw doses associated with field exposures are not available. Nonetheless and as discussed in Section 4.1.2.4.4, field and field simulation studies suggest that risks associated with contact exposures for bees can be reduced if contact is delayed for a period of at least 3 hours following application. These observations cannot be used to develop a formal exposure assessment but are discussed further in the risk characterization for terrestrial invertebrates (Section 4.4.2.4.1).

4.2.4. Terrestrial Plants

Terrestrial plants, particularly vegetation treated with spinosad, will certainly be exposed to any application that is effective in the control insect pests on the vegetation. Several different exposure assessments could be made for terrestrial plants, which are typically made for herbicides, including, direct spray, spray drift, runoff, wind erosion, and the use of contaminated irrigation water. For spinosad, however, the development of such exposure assessments would serve no purpose. As discussed in Section 4.1.2.5 (Hazard Identification for Terrestrial Plants), there is no evidence that spinosad will cause adverse effects in terrestrial plants. In the absence of an identified hazard, no formal exposure assessment is conducted for terrestrial plants.

4.2.5. Aquatic Organisms

An assessment of the effects of spinosad on aquatic organisms is based on estimated concentrations of spinosad in water that are identical to those used in the human health risk assessment (Section 3.2.3.4.6) and the risk assessment for terrestrial vertebrates (Section 4.2.2.4). The water contamination rates are summarized in Table 14, and the application of these rates to estimating expected concentrations of spinosad in water are discussed in Section 3.2.3.4.6.

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Overview

Table 31 provides an overview of the dose-response assessments used in the ecological risk assessment. The derivation of each of these values is discussed in the following subsections. Available toxicity data support separate dose-response assessments in seven groups of organisms: terrestrial mammals, birds, terrestrial invertebrates, fish, aquatic invertebrates, aquatic algae, and aquatic macrophytes. Separate dose-response assessments are developed for canids as well as non-canid mammals. In addition, separate dose-response assessments are developed oral and topical exposures of terrestrial invertebrates. No explicit dose-response assessments are justified for terrestrial plants, terrestrial or aquatic phase amphibians, and terrestrial macrophytes. Different units of exposure are used for different groups of organisms, depending on the nature of exposure and the way in which the toxicity data are expressed.

As with many insecticides, the most sensitive groups of organisms are terrestrial and aquatic arthropods. Based on estimates of acute oral NOAELs, the honeybee is more sensitive than mammals by a factor of over 9000 [370 mg/kg bw ÷ 0.041 mg/kg bw ≈ 9024] and more sensitive than birds by a factor of over nearly 5000 [200 mg/kg bw ÷ 0.041 mg/kg bw ≈ 4878]. For the current risk assessment, the lowest topical NOAEL for honeybees of 0.014 mg/kg bw is used to characterize risks associated with acute topical exposures. The topical toxicity values for bees, however, are highly variable and uncertainties associated with the characterization of risks associated with exposures are considered further in the risk characterization (Section 4.4.2.4.1).

As with terrestrial invertebrates, aquatic invertebrates are much more sensitive than aquatic vertebrates (i.e., fish) to spinosad. Based on chronic NOAECs for sensitive species, aquatic invertebrates are more sensitive than fish by a factor of almost 1000 [0.498 mg a.i./L ÷ 0.0005 mg a.i./L = 996]. Based on chronic NOAECs for tolerant species, aquatic invertebrates are more sensitive than fish by a factor of about 14 [1.15 mg a.i./L ÷ 0.0842 mg a.i./L ≈ 13.658]. Comparisons based on acute NOAECs are limited due to difficulties in identifying NOAECs for sensitive species of aquatic invertebrates. Some species of algae are more sensitive than fish with NOAECs of 0.05 mg a.i./L. Based on only a single bioassay in a species of duckweed, aquatic macrophytes appear to be tolerant of spinosad exposures—i.e., NOAEC of 1.86 mg a.i./L. The data are not sufficient to identify potentially sensitive species of aquatic macrophytes.

4.3.2. Toxicity to Terrestrial Organisms

4.3.2.1. Mammals

In characterizing risk to mammalian wildlife, Forest Service risk assessments generally use the NOAELs which serve as the basis for the acute and chronic RfDs from the human health risk assessment.

As discussed in Section 3.3.2, the surrogate acute RfD of 0.049 mg/kg bw is based on a NOAEL of 4.9 mg/kg from a 13-week assay of spinosad in dogs (MRID 43444102). The NOAEL of 4.9 mg/kg is used as the acute NOAEL for canids. As discussed in Section 3.3.3, the chronic RfD for spinosad is somewhat unusual in that the U.S. EPA/OPP/HED elected to derive the RfD for spinosad based on a chronic study on spinetoram in dogs with a NOAEL of 2.49 mg/kg bw/day (U.S. EPA/OPP/HED 2011a, MRID 47011901). Following standard practice in Forest

Service risk assessments, this chronic NOAEL is used as the basis for the chronic risk characterization in canids. As summarized in Appendix 1, Table A1-2, the use of the NOAEL 2.49 mg/kg bw/day for spinetoram is supported by a chronic NOAEL of 2.68 mg/kg/day for spinosad exposure in dogs of (Harada 1995, MRID 43701504).

The ecological risk assessment attempts to identify subgroups of organisms that may display greater or lesser sensitivity to a particular pesticide. These differences may be based on allometric scaling (e.g., Sample and Arenal 1999) or differences in physiology. As discussed in Section 3.1.5 and illustrated in Figure 5, dogs appear to be somewhat more sensitive than rodents to spinosad; furthermore, the greater sensitivity of dogs is reflected in the toxicity values for human health documented in U.S. EPA/OPP/HED (2011a) and used in the current Forest Service risk assessment. In the recent preliminary ecological assessment associated with the registration review of spinetoram, the acute LD_{50} of 3738 mg/kg bw is the lowest acute oral toxicity value cited (U.S. EPA/OPP/EFED 2011a, Table 11, pp. 27). As discussed in Section 3.1.4.1 and summarized in Appendix 1, Table A1-1, this is the lowest definitive LD_{50} value for spinosad (MRID 43414515). The Forest Service prefers to use NOAELs rather than LD_{50} values for risk characterization. In the absence of a NOAEL from this study, the LD_{50} is divided by 10 and rounded to two significant figures to approximate a NOAEL of 370 mg/kg bw. This approach to estimating a NOAEL from an LD_{50} is consistent with EPA's variable level-of-concern method, as detailed in SERA (2014a, Section 4.3.2).

For longer-term exposures in non-canid mammals, U.S. EPA/OPP/EFED (2011a, Table 11, pp. 28) identifies a NOAEL of 10 mg/kg bw/day citing MRID 43701506. As summarized in Appendix 1, Table A1-3, this MRID refers to a standard reproduction study in rats. This NOAEL is supported by a NOAEL of 9.5 mg/kg/day in male rats from a standard chronic toxicity study (Bond et al. 1995b, MRIDs 43701507 and 43710503). For the current Forest Service risk assessment, the NOAEL of 10 mg/kg bw/day is adopted from U.S. EPA/OPP/EFED (2011a). A previous EPA ecological risk assessments identifies a dietary NOAEL of >1100 ppm for MRID 43701506 (U.S. EPA/OPP/EFED 2009a, p. 40). While this information is provided for the sake of completeness, the more recent EPA documents cited above, reclassify the NOAEL as 10 mg/kg bw/day corresponding to a dietary concentration of 200 ppm.

4.3.2.2. Birds

As with mammals, Forest Service risk assessments generally defer to the U.S. EPA/OPP on study selection, unless there is a compelling reason to do otherwise. For characterizing risks to birds, U.S. EPA/OPP/EFED (2010a, Table 8, p. 25) uses an indefinite gavage LC_{50} of >1333 mg/kg bw in quail (Murray et al. 1992b, MRID 43414529, as summarized in Appendix 2, Table A2-1) to characterize risks associated with acute exposures. Based on the DER for this study, the NOAEL for quail is 200 mg/kg bw with a LOAEL of 500 mg/kg bw based on ataxia. Following the preference by the Forest Service to use NOAELs for risk characterization, the NOAEL of 200 mg/kg bw is used to characterize risks associated with acute exposures of birds to spinosad.

For chronic exposures, U.S. EPA/OPP/EFED (2010a, Table 8, p. 25) uses a dietary NOAEC of 550 mg/kg diet from standard reproduction studies in quail (Beavers et al. 1994a, MRID 43414533) and mallards (Beavers et al. 1994b, MRID 43414532). As summarized in Appendix 2, Table A2-2 of the current risk assessment, both of these studies yield LOAELs of 1100 mg/kg

diet based on embryotoxicity and decreased survival of offspring. As discussed in Section 4.2.2.3, the exposure assessment for birds is based on doses in units of mg/kg bw derived from food consumption estimates based on the concentration of the pesticide in food, the caloric values of different foods, and the caloric requirements of birds. Thus, the dietary NOAECs in units of mg/kg food are converted to doses in units of mg/kg bw. For both mallards and quail, dietary concentrations (mg/kg diet) are converted to mg/kg bw/day doses using a food consumption factor of 0.07 kg food/kg bw based on reproduction studies in quail and mallards (SERA 2007b). Using this food consumption factor, the dietary NOAEC of 550 mg a.i./kg diet corresponds to a dose of 38.5 mg/kg bw [550 mg/kg food x 0.07 kg food/kg bw].

4.3.2.3. Reptiles and Amphibians (Terrestrial Phase)

Since toxicity data are not available for terrestrial-phase reptiles or amphibians (Section 4.1.2.3), no dose-response assessment can be derived for this group of organisms.

4.3.2.4. Terrestrial Invertebrates

4.3.2.4.1. Contact Toxicity (bees)

The effects of direct spray or spray drift to terrestrial insects are typically assessed using the results of contact toxicity studies—i.e., studies in which the pesticide is applied by pipette to the insect. As discussed in Section 4.1.2.4.3, summarized in Table 19, and illustrated in Figures 6 and 7, contact toxicity assays are available on several species of terrestrial invertebrates. The LD_{50} values from the available studies are highly variable ranging from 0.025 mg/kg bw for the honey bee to 65 mg/kg bw for a bumblebee (*Bombus terrestris)*. This wide range spans a factor of 2600. Even within the assays on honeybees, the reported LD_{50} values range from 0.025 to 8.5 mg/kg bw, spanning a factor of about 340.

Consistent with the most recent EPA ecological risk assessment (U.S. EPA/OPP 2010a, p. 39), the lowest LD_{50} of 0.0029 mg/bee is from the study by Hoxter et al. (1992, MRID 43414547). The DER for this study indicates a NOAEL of 0.0016 µg a.i./bee, based on treatment-related mortality and signs of toxicity seen at higher doses. Taking a body weight of 116 mg for the honeybee (Table 18), this NOAEL is equivalent to a dose of about 0.014 mg/kg bw [0.0016 µg ÷ 0.116 g ≈ 0.01379 µg/g bw (mg/kg bw)].

While the NOAEL of 0.014 mg/kg bw is used in the EXCEL workbooks (Attachments 1 and 2) to calculate HQs for bees following contact exposure, the wide range of toxicity values discussed above is considered further in the risk characterization for terrestrial invertebrates (Section 4.4.2.4.1).

4.3.2.4.2. Oral Toxicity (bees)

The U.S. EPA risk assessments on spinosad do not explicitly derive toxicity values for oral exposures in bees and note that oral toxicity data for the honey bee was not submitted to the EPA (U.S. EPA/OPP/EFED 2011a, Table 14, p. 60). As summarized in Table 17 and detailed further in Appendix 3, Table A3-1, several acute oral LD_{50} values for honeybees are available in the open literature. The oral LD_{50} values in honeybees technical grade spinosad and spinosad formulations are remarkably consistent with a range from 0.41 to 0.54 mg/kg bw. As discussed in Section 4.3.2.1, the Forest Service prefers to use NOAELs rather than LD_{50} values for risk characterization. Adopting the EPA's variable level of concern method (SERA 2014a, Section

4.3.2), NOAELs may be approximated by dividing an acute LD_{50} by a factor of 10. In the absence of an acute NOAEL for honeybees, the lowest acute oral LD_{50} of 0.41 mg/kg bw (Carvalho et al. 2013) could be divided by 10 to approximate an acute NOAEL of 0.041 mg/kg bw.

Based on the contact toxicity data discussed in the previous section, dividing the oral NOAEL by a factor of 10 might be viewed as overly conservative. The contact assay by Hoxter et al. (1992, MRID 43414547) yields an LD_{50} of 0.029 mg/kg bw with an NOAEC of 0.014 mg/kg bw. The ratio of the NOAEL to the LD_{50} is 0.56 [0.014 mg/kg bw ÷ 0.0029 mg kg bw ≈ 0.013793]. Taking the acute oral LD_{50} of 0.41 mg/kg bw, the ratio of the contact NOAEL to contact LD_{50} could be used to estimate an oral NOAEL of 0.23 mg/kg bw [0.41 mg/kg bw x 0.56 = 0.2296 mg/kg bw].

Another alternative to using the standard factor of 10 to estimate an NOAEL from an acute LD_{50} could be based on the study by Barbosa et al. (2015). As discussed in Section 4.1.2.4.2, a dose of about 0.056 mg a.i./kg bw/day was associated with about 50% mortality in *Melipona quadrifasciata*, a species of stingless bee. The NOAEL for gross signs of toxicity (i.e., decreases in survival and pupal body weights as well as increases in the incidence of malformations) was about 0.012 mg a.i./kg bw/day. Based on this relationship, a factor of about 5 might be used to approximate the NOAEL [0.059 mg a.i./kg bw/day ÷ 0.012 mg a.i./kg bw/day ≈ 4.917].

A potential concern with using a factor of 5 based on gross signs of toxicity is the much lower NOAEC of 0.00059 mg/kg bw/day reported by Barbosa et al. (2015) based on abnormal walking activity in newly emerged adults. This NOAEL is a factor of 100 below the approximate LD_{50} [0.059 mg a.i./kg bw/day ÷ 0.00059 mg/kg bw/day = 100]. This relationship would suggest that the standard factor of 10 would be insufficient to estimate the NOAEL. The use of a factor of 100, however, could be viewed as overly conservative. As noted in the discussion by Barbosa et al. (2015):

> *To confirm the differences* [in walking behavior] *observed in the present study with bioinsecticide-exposed stingless bees, more complex experimental setups, including semifield and field studies, need to be performed. Such setups will allow the assessment of more complex behaviors, such as foraging, which are very important for colony survival.*

In other words, the concerns with pollinators are primarily focused on colony health. In the absence of confirming studies that would demonstrate an impact on colony health, the quantitative use of the very low NOAEL for walking behavior does not seem justified.

Given the above considerations, the current Forest Service risk assessment adopts the standard approach and estimates an NOAEL of 0.041 mg a.i./kg bw for the honeybee by dividing the lowest LD_{50} of 0.41 mg a.i./kg bw by a factor of 10. Reservations with this approach are considered further in the risk characterization (Section 4.4.2.4.3, Contaminated Nectar).

4.3.2.4.3. Oral Toxicity (phytophagous insects)

As summarized in Table 17 and discussed above, the acute oral LD_{50} values for honeybees are remarkably consistent, spanning a narrow range of 0.41 to 0.54 mg/kg bw with an average value

of 0.48 mg a.i./kg bw and a 95% confidence interval of 0.44 to 0.52 mg a.i./kg bw. As also summarized in Table 17, an LD_{50} of 0.23 mg/kg bw is available for spinosyn A in a phytophagous insect, the larvae of *Heliothis virescens* (tobacco budworm). This LD_{50}, which is lower than the estimated NOAEC of 0.3 mg/kg bw for the honeybee (Section 4.3.2.4.2), involved an injection exposure rather than an oral exposure. Because of this route of exposure as well as the nature of agent (i.e., spinosyn A rather than spinosad), the application of the LD_{50} to 0.23 mg/kg bw to the dose-response assessment for phytophagous insects seems questionable. On the other hand, as summarized in Table 17, lower oral LD_{50} values are available for *Bombus terrestris* (LD_{50} = 0.13 mg/kg bw) and a stingless bee, *Melipona quadrifasciata* (LD_{50} = 0.15 mg/kg bw). While these species are not phytophagous insects, the LD_{50} values support the assessment that in terms of oral exposure to spinosad, honeybees are not the most sensitive insect species.

For the current risk assessment, the lowest LD_{50} (i.e., 0.13 mg/kg bw for *Bombus terrestris*) is used as the basis for the dose response assessment for phytophagous insects. This approach is supported by the more extensive data on contact toxicity in insects (as illustrated in Figure 7 and discussed in Section 4.1.2.4.3.2) indicating that there are no substantial differences in sensitivity among Hymenoptera, Diptera, and Lepidoptera (an order of insects which are largely phytophagous). As with the approach used in estimating an oral NOAEL for honeybees (Section 4.3.2.4.2), the LD_{50} of 0.13 mg/kg bw for *Bombus terrestris* is multiplied by a factor of 0.64 and rounded to one significant place to estimate a NOAEC of 0.08 mg a.i./kg bw [0.13 mg/kg bw x 0.64 = 0.0832 mg/kg bw] for phytophagous insects.

The use of the 0.64 ratio derived from the contact toxicity data in the honeybee (Section 4.3.2.4.2.) is less well supported than the application of this factor to a phytophagous insect, because this application involves both route-to-route and species-to-species extrapolation. As discussed further in Section 4.4.2.4.2, the use of the 0.64 ratio rather than the default 0.1 ratio has no impact on the qualitative risk characterization for phytophagous insects—i.e., all HQs exceed the level of concern even at the lower bounds of exposures.

4.3.2.4.4. Soil Exposures (earthworms)

As discussed in Section 4.1.2.4.7, spinosad is not toxic to earthworms, and no definitive toxicity values are available. A well-documented 14-day NOAEL of 970 mg a.i./kg soil is available (U.S. EPA/OPP/EFED 2011a, p. 35, MRID 43414548). Forest Service risk assessments do not typically derive HQs for earthworms, and there is no reason to alter this practice in the current risk assessment. Based on the NOAEC of 970 mg a.i./kg soil, a qualitative characterization of risk is discussed briefly in Section 4.4.2.4.4.

4.3.2.5. Terrestrial Plants (Macrophytes)

No dose-response assessment is proposed for terrestrial plants. As discussed in Section 4.1.2.5, there is no basis for asserting that spinosad is likely to damage terrestrial plants. Risks to terrestrial plants are addressed semi-quantitatively in Section 4.4.2.5.

4.3.2.6. Terrestrial Microorganisms

As with terrestrial plants, there is little information available on the toxicity of spinosad to terrestrial microorganisms (Section 4.1.2.6). As discussed further in the risk characterization

(Section 4.4.2.6), the limited information that is available suggests that adverse effects in terrestrial microorganisms are not likely.

4.3.3. Aquatic Organisms

4.3.3.1. Fish

4.3.3.1.1. Acute Toxicity

As discussed in Section 4.1.3.1.1 and summarized in Appendix 5, Table A5-1, the available literature on spinosad includes several standard LC_{50} values in fish which the EPA uses to classify spinosad as slightly to moderately toxic to fish. While LC_{50} values are used directly in EPA risk assessments, the Forest Service prefers to use NOAECs in the dose-response assessment (SERA 2014a, Section 4.3). As noted in Appendix 5, Table A5-1, Data Evaluation Records (DERs as discussed in Section 1.2) are available for two species of fish, and the NOAECs for sublethal effects range from 1.8 mg a.i./L (sheepshead minnow with a corresponding LC_{50} of 7.87 mg a.i./L from York 1993) to 2.1 mg a.i./L (bluegill sunfish with a corresponding LC_{50} of 5.94 mg a.i./L from the study by Newsted and Brock 1992). While these are the best documented sublethal NOAECs, the LC_{50} values from these studies encompass a narrow range.

Of the reasonably well-documented LC_{50} values in fish, the lowest LC_{50} is 4.99 mg a.i./L in carp from the review by Cleveland et al. (2002b). The DER for the study by York (1993) yields the lowest ratio of NOAEC to LC_{50}—i.e., about 0.23 [1.8 mg a.i./L ÷ 7.87 mg a.i./L ≈ 0.2287]. This ratio is used to estimate an NOAEC for carp of about 1.1 mg a.i./L [4.99 mg a.i./L x 0.23 = 1.127 mg a.i./L], which is used to characterize risks in sensitive species of fish.

The highest definitive LC_{50} in fish is 30.0 mg a.i./L in rainbow trout from MRID 43444103, as summarized in both U.S. EPA/OPP/EFED (2011a) and Cleveland et al. (2002b). From the 0.23 ratio discussed above, the NOAEC for trout is estimated at 6.9 mg a.i./L [30 mg a.i./L x 0.23], which is supported by the NOAEC (normal swimming behavior) of 10 mg a.i./L in coho salmon from the study by Deardorff and Stark (2009). Deardorff and Stark (2009) also report an indefinite LC_{50} of >500 mg a.i./L in coho salmon. The somewhat lower estimated NOAEC of 6.9 mg a.i./L is used in the current risk assessment for tolerant species of fish.

4.3.3.1.2. Chronic Toxicity

As discussed in Section 4.1.3.1.2 and summarized in Appendix 5, Table A5-2, only two early life-stage studies were submitted to and accepted by the EPA: the standard early life stage studies in trout (Weinberg et al. 1993, MRID 43414541) and sheepshead minnow (MRID 44420601). The study in trout yields an NOAEC of 0.498 mg a.i./L, which is used to characterize risks associated with longer-term exposures in sensitive species of fish. The EPA uses this chronic NOAEC in trout to characterize longer-term risks to fish (U.S. EPA/OPP/EFED 2009a, p. 39). The early-life stage study in sheepshead minnow yields an NOAEC of 1.15 mg a.i./L, which is used to characterize risks associated with longer-term exposures in tolerant species of fish.

One reservation with this relatively standard and uncomplicated dose-response assessment for longer-term exposures of fish to spinosad involves the presumed sensitivity of trout. As discussed in the previous section, trout appear to be tolerant species in terms of acute exposures with an acute LC_{50} of 30 mg a.i./L and an estimated NOAEC of 6.9 mg a.i./L. The most sensitive species of fish in terms of acute toxicity is carp with a LC_{50} of 4.99 mg a.i./L and an estimated NOAEC of 1.1 mg a.i./L. In terms of acute toxicity, sheepshead minnow have an intermediate LC_{50} of 7.87 mg a.i./L and an experimental NOAEC of 1.8 mg a.i./L.

The lack of correspondence between sensitivities of different species of fish in acute and longer-term exposures is noted for the sake of clarity and transparency. While several different approaches using acute-to-chronic ratios could be used to estimate lower NOAECs for fish (NAS 2013), these approaches are typically reserved for addressing a lack of data rather than as an alternative to experimental data. From a practical perspective, reasonable applications of acute-to-chronic ratio methods would not have an impact on the risk characterization for longer-term exposures of fish to spinosad. As discussed further in Section 4.4.3.1, the HQs for fish are substantially below the level of concern.

4.3.3.2. Amphibians (Aquatic Phase)

No data are available on the toxicity of spinosad to aquatic phase amphibians (Section 4.1.3.2). Consequently, no dose-response assessment is developed for this group of organisms.

4.3.3.3. Aquatic Invertebrates

4.3.3.3.1. Acute Toxicity

4.3.3.3.1.1. Sensitive Species

As discussed in Section 4.1.3.3.1 and summarized in Table 25, data regarding the acute toxicity data of spinosad to aquatic invertebrates are more than ample. As with other groups of organisms, Forest Service risk assessments typically defer to EPA in terms of study selection for dose-response assessments, unless there is a compelling reason to do otherwise. In the case of spinosad, EPA considers some but not all of the relevant open literature (i.e., U.S. EPA/OPP/EFED 2011a, pp. 72-73). Specifically, the EPA does not consider or cite the study by Deardorff and Stark (2009) on three species of cladocerans. As summarized in Table 25, the study by Deardorff and Stark (2009) notes a 48-hour LC_{50} of 0.0018 mg a.i./L in a bioassay of a Success® formulation in *Ceriodaphnia dubia* [Cladocera: Daphniidae]. This LC_{50} is supported by several additional LC_{50} values in the range of 0.002 to 0.009 mg a.i./L in two families of Diptera—i.e., midge larvae [Diptera: Chironomidae, LC_{50} = 0.009 mg a.i./L], mosquito larvae [Diptera: Culicidae, LC_{50} = 0.002 mg a.i./L in *Anopheles stephensi*, LC_{50} = 0.0032 mg a.i./L in *Culex pipiens,* and LC_{50} = 0.007 in *Aedes aegypti*] (see Table 25 for details). For risk characterization in aquatic invertebrates, U.S. EPA/OPP/EFED (2009a, p. 39) uses the EC_{50} of 14 mg a.i./L in *Daphnia magna* from the study by Milazzo et al. (1994, MRID 43574502).

While the study from Deardorff and Stark (2009) is not specifically reviewed by EPA, it appears to have been well conducted and documented, is published in a peer reviewed journal (Journal of Environmental Science and Health, Part B), was conducted at an academic institution in the United States (Washington State University, Puyallup Research and Extension Center), and was funded by NOAA (National Oceanic and Atmospheric Administration). As discussed in Section 4.1.3.3.1, however, the study by Deardorff and Stark (2009) was conducted using a formulation

rather than the technical grade active ingredient (i.e., spinosad), and several comparisons of toxicity studies discussed in Section 4.1.3.3.1 suggest that the formulated products are more toxic than technical grade spinosad to aquatic invertebrates. As discussed in NAS (2013, p. 122), the use of toxicity data on formulations can be problematic if the formulation originates in a country other than the United States because foreign formulations may contain inerts which are not be used in U.S. formulations. This concern does not apply to the study by Deardorff and Stark (2009) who used a 240 g a.i./L Success® formulation from Dow AgroSciences, Indiana. As summarized in Table 3, the Forest Service specified that a 2 lb a.i./L (≈240 g a.i./L) Success® formulation would be a representative formulation for use in Forest Service programs.

Another concern with the use of formulation data involves environmental partitioning—i.e., the separation of inerts from the active ingredient over time due to differences in environmental fate characteristics of the components in the formulation, including the active ingredient. This consideration, however, affects the consideration of longer-term effects rather than acute effects (e.g., NAS 2013, pp. 121-122).

The above considerations constitute a compelling basis for differing from rather than deferring to the EPA. The dose-response assessment for sensitive species of aquatic invertebrates could be based on the LC_{50} of 0.0018 mg a.i./L in *Ceriodaphnia dubia*. Typically, a Forest Service risk assessment would divide the EC_{50} by a factor of 20 to approximate an acute NOAEL of 0.00009 mg a.i./L or 0.09 µg a.i./L [0.0018 mg/L ÷ 20] (SERA 2014a, Section 4.3.2, pp. 98-99). As discussed further in Section 4.3.3.3.2, however, a subsequent study by Deardorff and Stark (2011) determined a chronic NOAEC for *Ceriodaphnia dubia* of 0.5 µg a.i./L (0.0005 µg a.i./L). It would not be sensible to derive an acute NOAEC that is below the chronic NOAEC. As a possible alternative, the study in *Daphnia magna* used by EPA (i.e., Milazzo et al. 1994, MRID 43414537/43574502) report an EC_{50} of 14 mg a.i./L with a corresponding NOAEC of 0.883 mg a.i./L. The ratio of these values is about 0.063 [0.883 ÷ 14 ≈ 0.06307]. When multiplied by this ration, the LC_{50} of 0.0018 mg a.i./L in *Ceriodaphnia dubia* would estimate a NOAEC of about 0.00011 mg a.i./L or 0.11 µg a.i./L [0.0018 mg a.i./L x 0.063 ≈ 0.0001134 mg a.i./L]. This value is also below the chronic NOAEC 0.5 µg a.i./L reported by Deardorff and Stark (2011). Thus, in the absence of a viable alternative, the chronic NOAEC of 0.0005 mg a.i./L for *Ceriodaphnia dubia* is applied to acute exposures.

4.3.3.3.1.2. Tolerant Species

As summarized in Table 25, toxicity assays with aquatic invertebrates yielding EC_{50} values in excess of 1 mg a.i./L include three bioasays with *Daphnia magna* (Cleveland et al. 2002b; European Commission 2006; Milazzo et al. 1994, MRID 43574502), one bioassay with *Aedes aegypti* (Kovendan et al. 2012), one bioassay in an amphipod (MRID 47702901), and a bioassay in shrimp (Cleveland et al. 2002b). As discussed in Section 4.1.3.3.1, all of the studies yielding EC_{50} values greater than 1 mg a.i./L involve technical grade spinosad; formulations of spinosad appear to be much more toxic. As discussed in the previous section on sensitive species of aquatic invertebrates, the greater toxicity of spinosad formulations is relevant to the dose-response assessment for acute exposures.

As discussed in Section 4.1.3.3.1.2, several studies conducted in various species of mosquitos note substantial resistance to spinosad formulations. As with terrestrial insects (as summarized

in Table 24), very high resistance factors were observed in mosquitos following artificial selection pressure—e.g., the LC_{50} of 693.5 mg a.i./L in *Culex quinquefasciatus* (Su and Chen 2014b). These toxicity values are not considered for the dose-response assessment because the type of artificial selection pressure is not relevant to environmental exposures. Nevertheless, as summarized in Table 25, Su and Chen (2014b) report LC_{50} values of 0.196 to 0.460 mg a.i./L for a Natular® formulation in 3rd instar larvae of *Culex quinquefasciatus* from a wild population with no artificial selection pressure. A similar range of LC_{50} values—i.e., 0.234 to 0.424 mg a.i./L—is reported for a laboratory population of *Culex quinquefasciatus* with no prior exposure to spinosad. Consequently, it seems reasonable to use the upper bound LC_{50} of 0.460 mg a.i./L to represent relevant tolerant species/populations of aquatic invertebrates. Dividing this EC_{50} by a factor of 20 (SERA 2014a, Section 4.3.2, pp. 98-99) results in a NOAEL of 0.023 mg a.i./L [0.460 mg a.i./L ÷ 20].

4.3.3.3.2. Chronic Toxicity

The dose-response assessment for longer-term exposures to spinosad in aquatic invertebrates is relatively straightforward. As summarized in Table 26 and discussed in Section 4.1.3.3.2.1, daphnids and midges appear to be sensitive groups of aquatic invertebrates with most of the NOAECs spanning a relatively narrow range of 0.5 to 1.6 µg a.i./L. The only exception is the NOAEC of 8 µg a.i./L for a static renewal reproduction study in *Daphnia magna* reported by the European Commission (2006). The NOAEC of 1.2 µg a.i./L for a flow-through reproduction study in *Daphnia magna*, also reported in the review by European Commission (2006), is consistent with other NOAECs for daphnids and midges.

As discussed in Section 4.1.3.3.2.1, the LOAEC of 129 µg a.i./L in daphnids from the study by Stark (2005), which is summarized in Table 26, is not a reproduction study, and the high LOAEC simply reflects the study design—i.e., a single concentration substantially greater than the 48-hour EC_{50} with a nominal 10-day period of exposure.

Unlike the case with acute exposures of aquatic invertebrates, formulations of spinosad do not appear to be substantially more toxic than technical grade spinosad. This assessment is based primarily on the chronic bioassays in *Daphnia magna* using technical grade spinosad (MRID 43848801, NOAEC = 0.62 µg a.i./L, LOAEC = 1.2 µg a.i./L) and *Ceriodaphnia dubia* using a Success® formulation (Deardorff and Stark 2011, NOAEC 0.5 µg a.i./L, LOAEC = 1 µg a.i./L).

For the dose-response assessment of sensitive species of aquatic invertebrates involving longer-term exposures, the chronic NOAEC of 0.5 µg a.i./L in *Ceriodaphnia dubia* is used.

Based on the single chronic study in mysid shrimp (MRID 44420602), the NOAEC of 84.2 µg a.i./L is used for potentially tolerant species of aquatic invertebrates. While this is the only chronic study in this group of organisms, the full study was reviewed by EPA and classified as *Acceptable* (U.S. EPA/OPP/EFED 2011a, p. 26).

4.3.3.4. Aquatic Plants

4.3.3.4.1. Algae

As discussed in Section 4.1.3.4.1 and summarized in Appendix 7, Table A7-1, the toxicity data on the effects of spinosad are relatively uncomplicated. The most sensitive species is the

freshwater diatom, *Navicula pelliculosa* with an EC_{50} of 0.09 mg a.i./L and an NOAEC of 0.05 mg a.i./L (MRID 43414543). The NOAEC is used to characterize risks in sensitive species of algae.

In terms of both EC_{50} and NOAEC values, the most tolerant species is a green alga, *Selenastrum capricornutum*, with an indefinite EC_{50} of >105.5 mg/L and a corresponding NOAEC of 4.3 mg a.i./L (Cleveland et al. 2002b). The NOAEC of 4.3 mg a.i./L is used to characterize risks in tolerant species of algae.

4.3.3.4.2. Aquatic Macrophytes

As discussed in Section 4.1.3.4.1 and summarized in Appendix 7, Table A7-1, the only available study on aquatic macrophytes is a standard assay in duckweed (*Lemna gibba*) that reports an NOAEC of 1.86 mg a.i./L. In the absence of additional information, the assumption is made that duckweed is a tolerant species.

4.4. RISK CHARACTERIZATION

4.4.1. Overview

In the ecological risk assessment, as in the human health risk assessment, the quantitative expression of the risk characterization is the hazard quotient (HQ), the ratio of the anticipated dose or exposure to a no-observed-adverse-effect level or concentration (NOAEL/NOAEC) using 1 as the level of concern—i.e., an HQ of ≤ 1 is below the level of concern. The specific HQs discussed in this risk characterization are based on an application rate of 0.225 lb a.i./acre and encompass a single application (Attachment 1) or two applications with a 6-day application interval. The toxicity data and exposure estimates for spinosad support quantitative risk characterizations in mammals, birds, terrestrial insects (including pollinators), fish, aquatic invertebrates, and to a limited extent, aquatic plants. Risk characterizations for earthworms, soil microorganisms, and terrestrial plants are addressed qualitatively or semi-quantitatively (i.e., HQs are not derived) based on limitations in the available toxicity data. Risk characterizations are not developed for reptiles and amphibians due to the lack of toxicity data.

The organisms at greatest risk are the invertebrates, both terrestrial and aquatic. Adverse effects are virtually certain in sensitive species of phytophagous insects. Spinosad will be applied to terrestrial vegetation. Sensitive species of phytophagous insects that consume the contaminated vegetation will likely be killed. This risk characterization pertains to virtually any insecticide applied to vegetation at an effective application rate.

Potential risks to bees are also apparent but vary depending on the route of exposure. Honeybees as well as other insects that are directly sprayed with spinosad will probably be killed. A possible exception is *Bombus terrestris*, a species of bumblebee; however, data supporting the tolerance of this species are limited to a single study. In the absence of a replicate and confirming study, bumblebees are considered a group at potential risk following direct spray. Foliar interception of spinosad residues will substantially reduce risks to terrestrial insects. As a mitigating factor in risks to bees, the product labels for all formulations of spinosad indicate that the product should not be applied while bees are actively foraging. This limitation will substantially reduce risks to honeybees associated with direct spray or spray drift. The impact of these limitations on risks associated with foraging are less clear.

The HQs for foraging honeybees exposed to contaminated nectar are less than the HQs associated with direct spray; nonetheless, risks to foraging honeybees are substantial based on dose estimates associated with foraging for contaminated nectar. While there are substantial uncertainties with the exposure assessment presented in the current risk assessment, these uncertainties do not negate concerns for potential effects on honeybees and other pollinators via contaminated nectar following applications of spinosad. Most field or field simulation studies on risks to honeybees are not published in the open literature. Nonetheless, reasonably detailed reviews of these studies are available, and these field and field simulation studies do not indicate significant or substantial risks to foraging bees at application rates considered by the Forest Service. The available field studies are limited in that the studies are relatively short-term and focused on spray exposures rather than foraging. A field simulation study conducted over exposure periods of 3 to 5 weeks does raise concern for decreases in foraging activity at an

exposure equivalent to an application rate of about 0.07 lb a.i./acre. Longer-term field studies on colony health, including observations on colony overwintering, are not available.

Aquatic invertebrates, particularly sensitive species, could be at substantial risk following the application of spinosad in areas where the potential for water contamination is high, including areas with moderate to heavy rainfall. In arid areas, particularly areas with predominantly loam or sand soil textures, adverse effects on even sensitive species of aquatic invertebrates might not be observed. Given the variability in the estimated concentrations of spinosad in water, no general risk characterization for aquatic invertebrates is justified. In any site-specific application of spinosad, the risks will vary substantially with local conditions. Given the highly variable results from the generic water modeling used in the current risk assessment and the substantial impact that this variability has on the risk characterization for aquatic invertebrates, site-specific efforts to estimate surface water concentrations of spinosad might be justified, particularly in areas with moderate to heavy rainfall.

Vertebrates are less sensitive than invertebrates to spinosad. Nonetheless, foliar applications of spinosad could result in exposure levels that exceed the level of concern for some terrestrial mammals (longer-term exposures only) and birds (both acute and longer-term). For non-accidental exposure scenarios, risks to mammals and birds are associated with the consumption of contaminated vegetation, and risks are greatest for smaller animals consuming contaminated grasses or food items with spinosad concentrations comparable to those associated with contaminated grasses. The only HQ for accidental exposure scenario for terrestrial vertebrates that exceeds the level of concern is the upper bound HQ for a canid consuming contaminated fish. Except for an accidental spill scenario, risks to fish and aquatic vegetation appear to be insubstantial.

The risk characterization for spinosad focuses on the potential for direct toxic effects. Nonetheless, there is a potential for secondary or indirect effects in virtually all groups of nontarget organisms. Terrestrial applications of any effective insecticide, including spinosad, are likely to alter insect and other invertebrate populations within the treatment area. This alteration could have indirect effects on terrestrial or aquatic animals and plants, including changes in food availability, predation, and habitat quality. These indirect effects may be beneficial to some species and detrimental to others; moreover, the magnitude of indirect effects is likely to vary over time.

4.4.2. Terrestrial Organisms

4.4.2.1. Mammals

The quantitative risk characterization for mammals is summarized in Worksheets G02a of the EXCEL workbooks for a single application (Attachment 1) and two applications (Attachment 2). Based on central estimates of exposure, none of the exposure scenarios leads to HQs that exceed the level of concern (HQ=1). At the upper bounds of exposure, none of HQs for acute exposures exceeds the level of concern.

Only one upper bound HQ for accidental exposures exceeds the level of concern—i.e., an upper bound HQ of 17 for a canid consuming contaminated fish following an accidental spill. As discussed in Section 4.3.2.1, canids are considered a sensitive subgroup of mammals with a

NOAEL of 4.9 mg/kg bw/day and a corresponding LOAEL of 9.73 mg/kg bw/day from a subchronic study in dogs. The upper bound HQ of 17 is associated with a dose of about 83 mg/kg bw/day (Worksheet G01a of the attachments). This dose exceeds the LOAEL by a factor of about 9 [83 mg/kg bw/day ÷ 9.73 mg/kg bw/day ≈ 8.53]. As discussed in Section 3.3.2, the application of the subchronic study in dogs to acute single-dose exposures (i.e., this accidental exposure scenario) may be viewed as highly, perhaps overly, conservative. Acute/single-dose exposure studies in dogs are not available. Thus, the likelihood of observing frank adverse effects in canids consuming fish following an accidental spill is unclear.

In terms of chronic exposures, scenarios for the consumption of contaminated vegetation exceed the level of concern for both a single application (Attachment 1, upper bound HQs of 1.7 to 7). For two applications (Attachment 2), the upper bound HQs exceed the level of concerns for contaminated vegetation (HQs of 1.5 to 14) and contaminated fruit (an upper bound HQ of 1.2 for a small mammal. In all cases, the HQs are highest for small mammals.

The HQs for mammals are based on the assumption that 100% of the diet is contaminated (SERA 2014a, Section 4.2.2.3). This assumption may be unrealistic for some acute exposures and will probably be a rare event in terms of chronic exposures, at least for larger mammals (i.e., larger animals may move in and out of the treated areas). The impact of a limited consumption of contaminated vegetation based on less than 100% of the diet as contaminated is not considered quantitatively in the current risk assessment. Nonetheless, this consideration could be justified at least for some species in site-specific applications of spinosad.

4.4.2.2. Birds

The quantitative risk characterization for birds is summarized in Worksheet G02b of the EXCEL workbooks for a single application (Attachment 1) and two applications (Attachment 2). As with mammals, none of the central estimates of the HQs for birds exceeds the level of concern. In addition and as with non-canid mammals, the HQs for accidental exposure scenarios do not exceed the level of concern.

Several acute non-accidental and longer-term exposure scenarios for the consumption of contaminated vegetation exceed the level of concern for a small (10 g) bird but not for a larger (4 kg) bird. This pattern is similar to the pattern observed in mammals (Section 4.1.2.1) and reflects the greater food consumption of smaller birds relative to larger birds.

For acute exposures following a single application, the exceedances in the upper bound HQs are minor—i.e., an upper bound of 1.1 for contaminated broadleaf foliage and 1.9 for the consumption of short grass. For two applications, the exceedances in the upper bound HQs are about twice as high—i.e., an HQ of 2 for broadleaf vegetation and 4 for short grass. As discussed in Section 4.3.2.2, the acute LOAEC for birds is a factor of 2.5 higher than NOAEC (i.e., 500 mg/kg bw vs 200 mg/kg bw from the acute gavage study). Thus, the HQ of 4 for a small bird suggests that signs of toxicity might be observed. On the one hand, small birds typically do not consume large amounts of grasses in the vegetative stage; on the other hand, many birds consume significant amounts of grass seeds (USDA/NRCS 1999). Thus, concern for the scenario involving the consumption of contaminated grasses by small birds may be most relevant to contaminated grasses with seeds.

The upper bound HQs for longer-term exposures are somewhat higher than those for acute exposures. For single applications, the upper bounds of the HQs for the longer-term consumption of contaminated vegetation are 2 for tall grass, 3 for broadleaf vegetation, and 5 for short-grass. For two applications, the upper bounds of the HQs for the consumption of contaminated vegetation are 4 for tall grass, 5 for broadleaf vegetation, and 9 for short-grass. As discussed in Section 4.3.2.2, the longer-term LOAEC is a factor of 2 higher than the NOAEC (i.e., corresponding to an HQ of 2) based on embryotoxicity and decreased survival of offspring. While these effects should be viewed as severely adverse, concern is tempered by the lack of field studies reporting adverse effects in bird populations. This qualification may be important because all of the exposure scenarios for birds are based on the assumption that 100% of the diet is contaminated. As discussed in the previous section on mammals, this is a standard assumption used in all Forest Service risk assessments, which may in some cases grossly overestimate exposures in certain site-specific applications, particularly those in which spinosad is not broadcast over a wide area. These factors cannot be further considered in a generic assessment but could and should be considered quantitatively in site-specific assessments.

4.4.2.3. Reptiles and Amphibians (Terrestrial Phase)

No risk characterization is developed for reptiles or terrestrial phase amphibians because the available toxicity data do not support a dose-response assessment (Section 4.3.2.3).

4.4.2.4. Terrestrial Invertebrates

4.4.2.4.1. Direct Spray

The HQs for honeybees following direct spray and spray drift are summarized in Worksheet G09 of the EXCEL workbooks that accompany this risk assessment.

Spinosad is an effective insecticide, and the direct spray of a bee at an application rate of 0.225 lb a.i./acre leads to an HQ of 1103. This HQ is associated with a dose of about 15.4 mg/kg bw, which is above the lowest topical LD_{50} for bees of 0.025 mg/kg bw (Hoxter et al. 1992) by a factor of over 600 [15.4 mg/kg bw ÷ 0.025 mg/kg bw = 616]. As summarized in Table 19 and discussed in Section 4.1.2.4.3, there is a wide range of contact LD_{50} values for bees. Based on the highest estimated LD_{50} for honeybees of 8.5 mg a.i./kg bw (Mayes et al. 2003), the direct spray exposure is higher than the LD_{50} by a factor of about 2 [15.4 mg/kg bw ÷ 8.4 mg/kg bw ≈ 1.8333…]. These HQs suggest that the direct spray of a honeybee with spinosad at the application rate proposed by the Forest Service would be associated with substantial mortality in even tolerant populations of bees. As also summarized in Table 19, most other insects on which data are available, including other Hymenoptera, Diptera, and Lepidoptera, have contact LD_{50} values in the range of 0.5 to 2 mg a.i./kg bw. While exposure assessments are not quantified for these other groups of insects, it seems likely that they would be adversely affected by direct spray. By definition, this severe risk characterization for terrestrial insects is probably applicable to most insecticides applied at effective application rates.

The only noteworthy exception may involve the bumblebee. Based on the LD_{50} of about 65 mg/kg bw in *Bombus terrestris* estimated from data in the review by Mayes et al. 2003, it is not clear that all species of bumblebees would be adversely affected by direct spray. Substantial reservations with this speculation, however, involve the lack of confirming contact LD_{50} values

in bumblebees and the apparently greater sensitivity of *Bombus terrestris* relative to the honeybee based on oral toxicity, as summarized in Table 17.

As summarized in Worksheet G09, risks of contact exposures decrease substantially with increasing distance from the application site and increasing foliar interception. These are common observations with the application of any insecticide. Particularly for honeybees, application timing may be another substantial factor in mitigating risks. As noted in Section 4.1.2.4.1, all of the product labels contain language that should reduce the acute exposure of bees to spinosad during or shortly after application. In addition, as discussed in Section 4.1.2.4.4.2, the review by Mayes et al. (2003) cites unpublished field studies indicating that no signs of overt toxicity to bees were observed following evening applications of spinosad (i.e., when bees were not foraging). These studies, however, appear to have been relatively short-term and may not have accounted for the impact of exposures via foraging, which are discussed further in Section 4.4.2.4.3.

Lastly, incidents involving bee mortality associated with spinosad applications are not indicated in a review of incident reports to EPA. This reservation, however, is not viewed as a substantial factor in the risk characterization. As noted in the most recent EPA document on spinosad …absence *of reported incidents should not be construed as the absence of incidents* (i.e., U.S. EPA/OPP/EFED 2011a, pp. 39).

4.4.2.4.2. Consumption of Contaminated Vegetation

If spinosad is applied to vegetation at an effective rate, adverse effects on sensitive species of phytophagous insects are unavoidable. Given the use of spinosad to control damage to vegetation from phytophagous insects, this risk characterization is essentially a tautology. In addition, this severe risk characterization is to be expected given the higher sensitivity of insects to spinosad relative to mammals (Section 4.3.1) and the modest concerns in the risk characterization for some mammals consuming contaminated vegetation (Section 4.4.2.1).

The specific HQs for phytophagous insects are summarized in Worksheet G08b of the EXCEL workbooks that accompany this risk assessment. For a single application (Attachment 1), the lower bound HQs range from 5 to 51 depending on the type of vegetation consumed. The upper bound HQs range from over 90 to nearly 1500. For two applications (Attachment 2), the lower bound HQs range from 6 to 54 and the upper bound HQs range from 175 to over 2800.

As discussed in Section 4.3.2.4.3, there are some uncertainties in the dose-response assessment for phytophagous insects. The estimated NOAEC of 0.0832 mg/kg bw on which the HQs are based is derived from an oral LD_{50} of 0.13 mg/kg bw for *Bombus terrestris* rather than an injection LD_{50} of 0.23 mg/kg bw in the tobacco budworm. In addition, the approximation of the NOAEC is based on an adjustment factor of 0.64 rather than the more standard factor of 0.1. While these limitations are noted for the sake of transparency, they have no impact on the risk characterization. As summarized in Worksheet G08a, the HQs for broadleaf vegetation and small insects are based on doses of about 13 (2 to 69) mg/kg bw. The central estimate is higher than the highest oral LD_{50} for an insect (i.e., 2.7 mg/kg bw in the American cockroach as summarized in Table 17) by a factor of about 5. Similar comparisons may be made for two applications as well as other types of vegetation. In most cases, there is no doubt that an application of spinosad to vegetation at a rate of 0.225 lb a.i./acre will be detrimental to

numerous insects. As discussed in Section 4.1.2.4.5 and illustrated in Figure 9, adverse effects in several orders of insects are demonstrated in efficacy studies. As with the direct spray of a honeybee (Section 4.4.2.4.1), this risk characterization is essentially a tautology that is applicable to many insecticides. If an insecticide is applied to vegetation at an effective application rate, adverse effects, including substantial mortality, will occur in most insects with the possible exceptions of populations of insects resistant to spinosad.

As summarized in Table 24 and discussed in Section 4.1.2.4.6, resistance factors of up to about 2,000,000 are noted in insect populations subject to artificial selection pressure. These extreme resistance factors are probably not relevant to the risk characterization. Resistance factors of up to about 7000, however, are documented in field populations in the absence of artificial selection pressure. Even with the very high HQs discussed above for phytophagous insects, it seems reasonable to believe that some populations of resistant phytophagous insects might not be adversely affected. Again, however, this assessment is simply a restatement of the common problem that insects may develop resistance to otherwise effective insecticides unless prudent steps are taken (i.e., varying the types of insecticides applied) to minimize the development of resistance. This approach is essentially the motivator for organizations such as the IRAC Resistance Action Committee (IRAC 2016) as well as cautionary statements concerning resistance on the labels for most insecticides including spinosad.

4.4.2.4.3. Contaminated Nectar

The HQs for foraging bees are summarized in Worksheet G10 for one application at a rate of 0.225 lb a.i./acre (Attachment 1) and two applications at the same rate but with a 6-day application interval (Attachment 2). The HQs are 10 (5 to 22) for a single application and 15 (5 to 41) for two applications. As discussed in Section 4.3.2.4.2, the HQs are based on a NOAEC in honeybees of 0.041 mg/kg bw estimated from an oral LD_{50} of 0.41 mg/kg bw – i.e., the LD_{50} is a factor of 10 above the estimated NOAEC. Thus, the ratio of the exposures to the LD_{50} are 1 (0.5 to 2.2) for a single application and 1.5 (0.5 to 4.1) for two applications. Note that the similarity in the lower bound values of the ratios is attributable to the lower bound of the estimated half-life of spinosad in nectar (1.5 days) relative to the 6 day application interval (i.e., four half-lives.

Qualitatively, the risk characterization is unequivocal at the central estimates and upper bounds of the estimated exposures. These exposures reach or exceed the LD_{50}; thus, they could be associated with readily observable and perhaps substantial mortality in honeybees. At the lower bound of HQs, the estimated exposures are approximately one-half of the LD_{50}. As discussed in Section 4.3.2.4.2, doses associated with factors of 0.2 to 0.56 of the LD_{50} might not be associated with substantial or even observable rates of mortality. Nonetheless, as illustrated in the study by Barbosa et al. (2015), sublethal signs of toxicity (altered patterns of movement) could occur at doses substantially below the LD_{50} as well as NOAECs for gross signs of toxicity. Whether or not the sublethal effects on locomotion would be sufficiently severe to impact colony health is unclear.

While the oral toxicity data on honeybees are reasonably complete and consistent (Section 4.3.2.4.2), there are major uncertainties in the exposure assessment (Section 4.2.3.3). Specifically, there are no monitoring studies on the levels of spinosad in nectar; hence, the concentrations are approximated using monitoring data for spinosad in pollen (Bailey et al. 2005)

along with empirical relationships between pesticide concentrations in pollen and nectar (Dively and Kamel 2012). In addition, the lack of data on the kinetics of spinosad in nectar and pollen adds uncertainty to the assessment of potential exposures from multiple applications and estimates of the length of time that risks associated with spinosad in nectar might persist.

As summarized in Table 22 and discussed in Section 4.1.2.4.4.2, the above risk characterization for foraging honeybees has only limited support from the available field and field simulation studies. At application rates in the range of about 0.2 lb a.i./acre, an increase in honeybee mortality was observed; however, it is not clear that the increases were statistically significant (Mayes et al. 2003, citing studies by Kirkland 1999 and Halsall 2002). At an application rate of 0.48 lb a.i./acre (roughly equivalent to two applications at 0.225 lb a.i./acre), increased mortality was evident but not apparently statistically significant; nonetheless, a reduction was observed in brood development (Mayes et al. 2003 citing Vinall 2000).

As also summarized in Table 22 and further detailed in Appendix 3, Table A3-4, several field and field simulation studies indicate that no adverse effects were demonstrated in honeybees at application rates of ≤0.16 lb a.i./acre. In reviewing these studies, Miles et al. (2011) note:

> *Assessments performed up to 7 days after treatment made during bee activity confirm the absence of mortality to foragers visiting treated flowers at 96 g a.s./ha* [≈0.085 lb a.i./acre]. *Therefore, this exposure rate can be considered as a threshold for immediate acute toxicity, but at which no long lasting acute toxicity is expected at this application rate or higher.*
>
> Miles et al. 2011, p. 113

In some respects, the suggestion that 0.085 lb a.i./acre may be a *threshold* for acute toxicity seems overly conservative. As noted above and detailed in Appendix 3, field studies at or below 0.16 lb a.i./acre have not demonstrated acute adverse effects in honeybees. Nonetheless, accepting the above estimate of 0.085 lb a.i./acre as a functional NOAEL based on a field study, the application rate of 0.225 lb a.i./acre proposed by the Forest Service corresponds to an HQ of about 3 [0.225 lb a.i./acre ÷ 0.085 lb a.i./acre ≈ 2.6].

Substantial reservations with the available field studies involve the durations of exposure and durations of observation. As summarized in Table A3-4, most of the available field studies involve relatively brief periods of observation (1 to several days) that are focused more on the impact of direct spray rather than exposures through foraging. The longest term field or field simulation study is published in the paper by Morandin et al. (2005) and involved foraging by bumblebees on artificial flowers over exposure periods of 3 to 5 weeks. Adverse effects noted in this study included decreased levels of activity and trembling during foraging; moreover, these effects occurred in exposures equivalent to application rates of about 0.07 lb a.i./acre, below the presumptive NOAEL of 0.085 lb a.i./acre from Miles et al. (2011), as discussed above. As discussed in Section 4.1.2.4.2, decreased activity was also observed in a species of stingless bee at doses substantially below the LD_{50} in the study by Barbosa et al. (2015). The lack of longer-term field studies, particularly studies involving colony overwintering, is a concern. As discussed in the recent Forest Service risk assessment on imidacloprid (SERA 2015), longer-

term studies on the overwintering of bee colonies can provide sensitive endpoints for assessing the impact of pesticides on pollinators.

The above risk characterization for foraging is focused on the honeybee because the exposure assessment developed in the current risk assessment is based on published exposure assessment methods for the honeybee – i.e., Alix and Vergnet (2007), Halm et al. (2006), and Rortais et al. (2005) as detailed in Section 4.2.3.3.1. Nonetheless, as summarized in Table 17 and discussed in Section 4.1.2.4.2, acute oral toxicity studies in a species of bumblebee (*Bombus terrestris* , Mayes et al. 2003) and a species of stingless bee (*Melipona quadrifasciata*, Tom et al. 2015) suggest that these bees may be more susceptible than honeybees to spinosad. In addition, the study by Barbosa et al. (2015) indicates that sublethal effects may occur in *Melipona quadrifasciata* at doses substantially below those associated with gross signs of toxicity such as mortality and reduced growth. While the studies on bees other than the honeybee are not used quantitatively in the current risk assessment, these studies raise concern that adverse effects may occur in other species of bees in addition to the honeybee.

The EPA did not conduct a risk assessment for foraging honeybees. In a recent ecological risk assessment, the EPA notes: *Because spinosad is toxic to honeybees, risk is assumed* (U.S. EPA/OPP/EFED 2009a, p. 48). On the other hand, the more recent ecological assessment of spinosad in support of the registration review expresses little concern for the contamination of pollen following foliar application: *Systemicity of spinosad into plant tissue, including possible contamination of pollen and nectar related to pollinator health, does not appear to be a route of concern considering that the majority of uses are foliar applications* (U.S. EPA/OPP/EFED 2011a). As summarized in Section 4.2.3.3.2, the study by Bailey et al. (2005), which provides the residue data for spinosad in pollen, involved foliar application. While U.S. EPA/OPP/EFED (2011a, p. 69) cites the study by Bailey et al. (2005), the residue data from the study are not discussed in the EPA document. The analysis presented in the current Forest Service risk assessment differs from the EPA's assessment that the potential contamination of nectar and pollen …*does not appear to be a route of concern*. As discussed in Section 2.2, spinosad is labelled for and will be applied in broadcast applications. In broadcast applications, nontarget plants that might be a source of nectar or pollen for honeybees may be contaminated. Based on the exposure assessment and dose-response assessment in the current Forest Service risk assessment, adverse effects including mortality in honeybees are plausible. While there are substantial uncertainties with the exposure assessment presented in the current risk assessment, these uncertainties do not negate concerns for potential effects on honeybees and other pollinators following applications of spinosad.

4.4.2.4.4. Soil Exposures

As discussed in Section 4.2.3.4 and summarized in Table 30, the maximum estimated soil concentration rates for spinosad are 0.38 mg a.i./kg soil per lb a.i./acre for a single application and 0.82 mg a.i./kg soil per lb a.i./acre for two applications. Adjusted for the application rate used in the current risk assessment, 0.225 lb a.i./acre, the maximum expected concentrations in soil are about 0.09 mg a.i./kg soil for a single application [0.38 x 0.225 = 0.0855] and 0.18 mg a.i./kg soil for two applications [0.82 x 0.225 = 0.1845]. As noted in Section 4.3.2.4.4, the NOAEC for earthworms is 970 mg a.i./kg soil based on a study summarized in EPA (U.S. EPA/OPP/EFED 2011a, p. 35, MRID 43414548). This NOAEC is above the highest estimated concentration of spinosad in soil by a factor of over 5000 [970 mg a.i./kg soil ÷ 0.18 mg a.i./kg

soil ≈ 5388.89]. Based on the much higher NOAEC relative to anticipated concentrations of spinosad in soil, there is no reason to expect that spinosad will cause adverse effects in earthworms.

4.4.2.5. Terrestrial Plants

No quantitative risk for terrestrial plants is proposed. As discussed in Section 4.1.2.4, there is no indication in the standard Tier 1 phytotoxicity studies reviewed by the EPA of adverse effects on terrestrial plants at an application rate of 0.5 lb a.i./acre; furthermore, this application rate is substantially above that proposed by the Forest Service (i.e., 0.225 lb a.i./acre). Moreover, as documented in the open literature, spinosad was tested extensively in both laboratory and field studies for its efficacy in protecting terrestrial plants from insect pests. If spinosad were toxic to plants at applications rates used to control the pest species, the available data would most likely include detailed published reports of phytotoxicity.

4.4.2.6. Terrestrial Microorganisms

As with earthworms (Section 4.4.2.4.4), only limited information is available on the toxicity of spinosad to terrestrial microorganisms. Based on studies briefly summarized in the the European Commission (2006) review, adverse effects were not observed on nitrogen or carbon mineralization by soil microorganisms at spinosad concentrations of 0.72 mg a.i./kg soil. This NOAEC is above the maximum expected concentration of spinosad in soil (i.e., 0.19 mg a.i./kg soil as discussed in Section 4.4.2.4.4) by a factor of about 4 [0.72 mg/kg soil ÷ 0.19 mg a.i./kg soil ≈ 3.789].

4.4.3. Aquatic Organisms

4.4.3.1. Fish

The HQs for fish are summarized in Worksheet G03 of Attachment 1 (one application) and Attachment 2 (two applications). The risk characterization for fish is reasonably simple and unequivocal. Based on expected levels of exposure (excluding accidental exposures), none the HQs for fish exceeds the level of concern. The highest HQ is 0.07, the upper bound HQ for sensitive species of fish based on acute exposure following two applications. This HQ is below the level of concern by a factor of about 14 [1 ÷ 0.07 ≈ 14.286]. Given the broad range of conditions used to estimate expected concentrations of spinosad in surface water (Section 3.2.3.4.3), direct toxic effects on fish following applications anticipated in Forest Service programs or related activities would seem implausible.

In the case of an accidental spill, the upper bound HQ for sensitive species of fish is 4. As summarized at the top of Worksheet G03, this HQ is associated with a concentration of about 4.1 mg a.i./L. As discussed in Section 4.1.3.1.1, the lowest LC_{50} for fish is 4 mg a.i./L. Based on this relationship, the accidental spill modeled for the current risk assessment (Section 3.2.3.4.1) would be expected to cause detectable and perhaps substantial levels of mortality in sensitive species of fish. Whether or not an actual spill would cause fish mortality depends on the amount of spinosad released into the water and the characteristics of the waterbody, including size and water turnover or flow rates, and the sensitivities of the fish populations in the affected area.

As discussed in the following section, adverse effects on at least some aquatic invertebrates are likely. Consistent with the conclusions in a previous EPA risk assessment (U.S.

EPA/OPP/EFED 2009a, p. 55), indirect effects on fish are possible due to direct adverse effects on aquatic invertebrates—e.g., reduced food supply.

4.4.3.2. Amphibians (Aquatic Phase)

Because toxicity data on aquatic phase amphibians are not available, no explicit risk characterization is developed for this group of organisms. The recent EPA assessment of spinosad (U.S. EPA/OPP/EFED 2011a, p. 47) recommends the use of fish as a surrogate for aquatic phase amphibians. This is a standard practice in EPA ecological risk assessments.

4.4.3.4. Aquatic Invertebrates

The HQs for aquatic invertebrates are summarized in Worksheet G03 of the EXCEL workbooks which accompany this risk assessment—i.e., Attachment 1 for a single application and Attachment 2 for two applications. The risk characterizations for both application scenarios are similar.

In terms of peak/acute expected concentrations in water, the HQs bracket the level of concern. For sensitive species, the HQs are 3 (0.04 to 77) for a single application and 6 (0.05 to 153) for two applications. For tolerant species, the HQs are 0.07 (0.0008 to 1.7) for a single application and 0.1 (0.001 to 3) for two applications. These broad ranges of HQs reflect the wide-range of conditions (i.e., temperature, rainfall, and soil textures) used in the GLEAMS-Driver modelling on which the exposure assessments are based (Section 3.2.3.4.3). Qualitatively, the HQs suggest that it is unlikely that tolerant species of invertebrates would be adversely affected. For sensitive species, however, the risk characterization is indefinite. In areas with a low potential for water contamination, no adverse effects on even sensitive species of aquatic invertebrates might be observed. In areas with a higher potential for water contamination, adverse effects and probably substantial mortality would be noted in sensitive species of aquatic invertebrates.

In terms of longer-term risks to aquatic invertebrates, the risk characterization for sensitive species is similar to that for acute exposures. The longer-term HQs are 1.1 (0.007 to 30) for a single application and 2 (0.01 to 59) for two applications. For tolerant species, the chronic HQs are below the level of concern even at the upper bounds of exposure. The highest HQ for tolerant species is 0.3—i.e., the upper bound of the HQ tolerant species following two applications.

The high variability in the estimated concentrations of spinosad in water precludes a general risk characterization. In any site-specific application of spinosad, the risks will vary substantially with local conditions. Further guidance on the variability in the concentrations of spinosad in water can be gleaned from Appendix 8 (one application) and Appendix 9 (two applications). For example, Table A8-7 gives the expected water contamination rates following a single application. Relatively arid areas, particularly those with predominantly loam or sandy soil textures, have the lowest water contamination rates. Much higher water contamination rates are evident in areas with moderate or substantial rainfall. The specific average annual rainfalls for the nine locations used in the modeling are listed in Table 10. Given the highly variable results from the water modeling used in the current risk assessment and the substantial impact that this variability has on the risk characterization for aquatic invertebrates, site-specific efforts to estimate concentrations of spinosad in surface water might be justified, particularly in areas with moderate to heavy rainfall.

In the case of an accidental spill, the risk characterization is simple and unequivocal. As summarized at the top of Worksheet G03, the accidental spill scenarios estimate concentrations of spinosad in water of about 1 (0.1 to 4) mg a.i./L. As summarized in Table 25 and discussed in Section 4.1.3.3.1, most LC_{50} values for spinosad formulations in aquatic invertebrates range from 0.0018 mg a.i./L to about 0.09 mg a.i./L. In the event of an accidental spill, the likelihood of extensive mortality in many species of aquatic invertebrates is virtually certain. Substantial mortality after an accidental spill is likely in even the most tolerant species—i.e., mosquitoes with EC_{50} values in the range of 0.2 to about 0.5 mg a.i./L. As is true in the accidental spill scenario for fish (Section 4.4.3.1), the extent of mortality among aquatic invertebrates following an actual spill would depend on the amount of spinosad released into the water and the characteristics of the waterbody, including size and water turnover or flow rates.

4.4.3.4. Aquatic Plants

The risk characterization for algae and aquatic macrophytes is reasonably simple. While this group of organisms has not been studied as extensively as fish and aquatic invertebrates, there is no indication that aquatic plants will be adversely affected by concentrations of spinosad in surface water, even considering the very broad range of estimated concentrations for various locations and climates. The only exceedance in the level of concern (HQ=1) is the upper bound HQ of 1.5 for sensitive species of algae based on peak estimates of exposure following two applications. There is no basis for asserting that this modest exceedance would lead to detectable changes in the algal community.

In the event of an accidental spill, the HQs for sensitive species of algae—i.e., 20 (2 to 82)—clearly indicate the potential for adverse effects. This potential is similar to (albeit less extreme than) the anticipated effects on aquatic invertebrates following an accidental spill (Section 4.4.3.4).

5. REFERENCES

NOTE: The initial entry for each reference in braces {} simply specifies how the reference is cited in the text. The final entry for each reference in brackets [] indicates the source for identifying the reference.

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FS	**Documents from Forest Service personnel.**
Imid	**From 2015 imidacloprid risk assessment.**
Sec	**Study summarized from a secondary source.**
Set00	**Papers from preliminary scoping and incidental searches.**
Set01	**Papers from ECOTOX and initial TOXLINE screen.**
Set02	**Supplemental studies on environmental fate.**
Set03	**Supplemental studies on insects.**
Set04	**Additional background material and search for nectar residues.**
Set05	**Additional toxicity values from ECOTOX.**
Set06	**Post peer review update search and papers from peer reviewers.**
Std	**Standard references used in most Forest Service risk assessments.**

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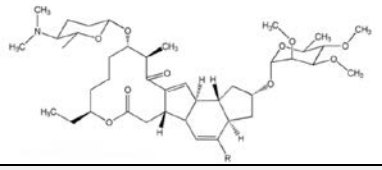
Table 1: Summary of Open Literature

Topic	Citations
Human Health	
Dermal Effects	McCormack 2011
General	El-Hoda et al. 2012; Kanoh and Rubin 2010 ;
Carcinogenicity	Aciole et al. 2014; Akmoutsou et al. 2011; Stebbins et al. 2002; Yano et al. 2002
Pharmacokinetics	Dunn et al. 2011; Holmstrom et al. 2012; Mackay et al. 2012; Rothwell et al. 2005 [sheep]
Reproductive Effects	Breslin et al. 2000; Hanley et al. 2002; Marty et al. 1998; Uggini et al. 2012
Veterinary Toxicology	Beugnet et al. 2011; Franc and Bouhsira 2009; Elanco 2012; Paarlberg et al. 2013; Schrickx 2014; Sherman et al. 2010; Snyder et al. 2013; Wolken et al. 2012
Human Data	Cole and Lundquist 2011; Gunning et al. 2012; Shmidt and Levitt 2012; Stough 2012; Stough et al. 2009 [all medicinal]; Su et al. 2011 [poisoning]
Dietary Exposure	Gao et al. 2007b
Terrestrial Species	
Birds	George et al. 2010; Magnussen et al. 1996; Uggini et al. 2012
Bees	Besard et al. 2011; Cabrera-Mar¡n et al. 2015; Carvalho et al. 2013; Gomez-Escobar et al. 2014; Mangan and Moreno 2009; Mayes et al. 2003 [Review]; Miles 2003; Miles et al. 2002; Miles et al. 2011; Morandin et al. 2005; Rabea et al. 2010; Sanchez et al. 2012; Scott-Dupree et al. 2009; Tom et al. 2015
Insect, general	Amarasekare and Edelson 2004; Hussain et al. 2009; Khan and Akram 2014; Musser and Shelton 2005; Rinkevich and Scott 2013; Salgado 1998; Salgado et al. 1998; Schneider et al. 2003
Insects, non-target	Beloti et al. 2015; Baur et al. 2003; Benamu et al. 2007, 2013; Biondi et al. 2012, 2013; Brunner et al. 2001; Cisneros et al. 2002; de Freitas Bueno et al. 2008; Eelen et al. 2006; Elzen and Elzen 1999; Elzen et al. 1999; Holt et al. 2006; Jones et al. 2005; Lawler and Dritz 2013;Liu and Zhang 2012; Liu et al. 2013a; Martinou et al. 2014; Michaud 2003; Miles and Eelen 2006; Muddasir et al. 2015; Naveed et al. 2008; Nowak et al. 2001; Pietrantonio and Benedict 1999; Rahman et al. 2011; Rimoldi et al. 2012; Schneider et al. 2003; Schneider et al. 2004; Schoonover and Larson 1995; Stark et al. 2004; Studebaker and Kring 2003; Thomas and Mangan 2005; Villanueva and Walgenbach 2005; Wang and Messing 2006; Wang et al. 2012; Williams and Price 2004; Williams et al. 2003. [Some may involve aquatic larvae.]
Efficacy	Many publications. See Section 4.1.2.4.5 for discussion.
Insect Resistance	Bao et al. 2014; Bielza et al. 2007, 2008; Campos et al. 2014; Gao et al. 2007a; Hou et al. 2014; Hsu and Feng 2006; Hsu et al. 2012a,b; Hsu et al. 2012a,b; Huang et al. 2004; Khan et al. 2011; Liu et al. 2004a,b; Markussen and Kristensen 2012; Sagri et al. 2014; Sayyed et al. 2008; Scott 1998; Shi et al. 2011; Su and Cheng 2014a,b; Zhang et al. 2014. Several additional studies
Plants	Haile et al. 1999
Aquatic Species	
Fish	Anogwih et al. 2013; Elskus 2007; Piner and Šner 2013; Piner and Uner 2012, 2014
Invertebrates, Aquatic.	Antonio et al. 2008; Cetin et al. 2005; Darriet et al. 2005 ; Deardorff and Stark 2009, 2011; Duchet et al. 2008, 2010a,b, 2011, 2015; Infante-Rodríguez et al. 2011; Jiang and Mulla 2009; Jones and Ottea 2013; Kovendan et al. 2012; Kumar et al. 2011; Mansour et al. 2012; Marina et al. 2012, 2014; Perez et al. 2007; Pridgeon et al. 2008; Romi et al. 2006; Stark and Vargas 2003; Su et al. 2014; Tome et al. 2014; [Includes several studies on mosquito larvae as target species.]
Other	
Environmental Fate and Properties	Soil: Hale and Portwood 1996; Sharma et al. 2007; Thompson et al. 2002a,b; Fruit/Vegetation: Berard and Santonin 1996; Liu et al. 2013b; Kovacova et al. 2013; Mandal et al. 2009, 2013; Santis et al. 2012; Sharma et al. 2007; Vijayasree et al. 2014; Water: Cleveland et al. 2002a; Liu and Li 2004; Perez et al. 2007
Forestry Efficacy	Cranshaw et al. 2014; Harrell and Stepanek 2005; Lewis et al. 2007; Nebraska Forest Service 2009; Nowak et al. 2000, 2001, 2010; Peusens and Belian 2012; Semiz et al. 2006; Thompson et al. 2002a,b; Wanner et al. 2002
Reviews	Cleveland et al. 2002a,b; Dow 2014; Dow Elanco 1996; Elanco 2012; EFSA 2011, 2012, 2013, 2014; FAO/WHO 2001; Gao et al. 2007b; HSDB 2003 [Spinosyn-A only]; Kirst et al. 1992; Mandal et al. 2013; Mayes et al. 2003; McCormack 2011; McFadden and Saunders 2004; Sparks et al. 1998; Thompson et al. 2015; USDA/APHIS 1999, 2003, 2011, 2014; WHO 2008, 2011. Many EPA documents (Section 5).

See Section 1.1 for discussion.

Table 2: Chemical and Physical Properties

Item	Value	Reference[1]
	Identifiers	
Common name	Spinosad	
Composition	Spinosyn A (dominant) Spinosyn D (minor)	Dow Elanco 1996
CAS Name	See ChemIDplus 2015a,b,c	
CAS No.	Spinosad: 168316-95-8 Spinosyn A: 131929-60-7 Spinosyn D: 131929-63-0	ChemIDplus 2015a,b,c Dow 2014; European Commission 2006
Development Codes	XDE-105 (90.4% a.i.)	Tomlin 2004; EPA/OPP/HED 1997b
	DE-105 (Dow)	Tomlin 2004; U.S. EPA/OPP/HED 1997b
	NAF-144 (technical end-use product, 2.6% a.i.)	EPA/OPP/HED 1997b
	NAF-85 (Tracer® formulation, 44.2% a.i.)	EPA/OPP/HED 1997b
IUPAC Name	See ChemIDplus 2015a,b,c	
IRAC Resistance Category	5	IRAC 2015 Sparks and Nauen 2015
Molecular formula	Spinosyn A: $C_{41}H_{65}NO_{10}$ Spinosyn D: $C_{42}H_{67}NO_{10}$ These are the correct formulae.	Tomlin 2004 HSDB 2013 (Spinosyn A) U.S. EPA/OPP/EFED 2011a
	Spinosyn A: $C_{41}H_{65}NO_{16}$ Spinosyn D: $C_{42}H_{67}NO_{16}$ Error in number of oxygens.	Dow Elanco 1996 Thompson et al. 2015
	Spinosyn A: $C_{42}H_{67}NO_{16}$ Spinosyn D: $C_{41}H_{65}NO_{16}$ Error in number of carbons (A and D switched) and oxygens.	Thompson et al. 2015
Mechanistic group	Nicotinic acetylcholine receptor (nAChR) allosteric activator. Included with spinetoram.	IRAC 2015
	A different site from nicotine or imidacloprid.	Tomlin 2004
Smiles Code with stereochemistry	See ChemIDplus 2015a,b,c	
Structure		Kirst et al. 1992 and several later sources. See Figure 1 for details.
	Chemical Properties(1)	
Aqueous photolysis	Spinosyns A and D (2 ppm): half-lives of 0.8-0.9 days in pH 7 buffer, sunlight, 25 ± 1°C, for 48 hour observation. **Working Note: Spinosad assumed to be stable for PRZM/EXAMS and GENEEC2 in EFED 2009a drinking water assessment.**	MRID 43507302 U.S. EPA/OPP/EFED 2005
	Spinosyns A and D (2 ppm): half-lives of 0.54-0.55 days, pond water, pH 9.2, sunlight, 25 ± 1°C, for 48 hour observation.	MRID 44597735 U.S. EPA/OPP/EFED 2005

Item	Value	Reference[1]
K_{ow}	Spinosyn A ≈631 [logP = 2.8 (pH 5)] 10,000 [logP = 4 (pH 7)] ≈158,000 [logP = 5.2 (pH 9)] Spinosyn D ≈1,600 [logP = 3.2 (pH 5)] ≈31,000 [logP = 4.5 (pH 7)] ≈158,000 [logP = 5.2 (pH 9)]	Dow Elanco 1996; Tomlin 2004; Thompson et al. 2015; U.S. EPA/OPP/HED 2011a
	Spinosyn A ≈603 [logP = 2.78 (pH 5)] 10,200 [logP = 4.01 (pH 7)] ≈145,000 [logP = 5.16 (pH 9)] ≈8,130 [[logP = 3.91 (distilled water)]] Spinosyn D ≈1,700 [logP = 3.23 (pH 5)] ≈33,900 [logP = 4.53 (pH 7)] ≈162,000 [logP = 5.21 (pH 9)] ≈240 [[logP = 2.38 (distilled water)]]	U.S. EPA/OPP/EFED 2011a
Molecular weight (g/mole)	Spinosyn A: 731.98 Spinosyn D: 746	Dow Elanco 1996; Tomlin 2004; Thompson et al. 2015.
	Spinosyn A: 731.976 Spinosyn D: 745.998	U.S. EPA/OPP/EFED 2011a
Melting point	Spinosyn A: 84-99.5 °C Spinosyn D: 161.5-170 °C	Dow Elanco 1996; Tomlin 2004; Thompson et al. 2015.
pKa	Spinosyn A: 8.1 Spinosyn D: 7.87	U.S. EPA/OPP/EFED 2011a U.S. EPA/OPP/HED 1997b
Vapor pressure	Spinosyn A: 3.0×10^{-5} mPa (25 °C) Spinosyn D: 2.0×10^{-5} mPa (25 °C)	Tomlin 2004; U.S. EPA/OPP/HED 1997b
	Spinosyn A: 2.4×10^{-10} mg Hg Spinosyn D: 1.5×10^{-10} mg Hg Note: These values are identical to the values given in Tomlin 2004. Difference is in units. 1 mPa = 0.0000075 mg Hg.	Dow Elanco 1996; Thompson et al. 2005
	Spinosyn A: 2.4×10^{-10} mg Hg Spinosyn D: 1.6×10^{-10} mg Hg	U.S. EPA/OPP/EFED 2011a
Water solubility	Spinosyn A: 89 mg/L (distilled water, 20 °C) 235 mg/L (pH 7, 20 °C) Spinosyn D: 0.5 mg/L (distilled water, 20 °C) 0.33 mg/L (pH 7, 20 °C)	Tomlin 2004

<table>
<tr><th>Item</th><th>Value</th><th>Reference[1]</th></tr>
<tr><td></td><td>Buffered water
<table>
<tr><th>pH</th><th>Spinosyn A (mg/L)</th><th>Spinosyn D (mg/L)</th></tr>
<tr><td>5</td><td>290</td><td>29</td></tr>
<tr><td>7</td><td>235</td><td>0.332</td></tr>
<tr><td>9</td><td>16</td><td>0.053</td></tr>
</table>
Distilled water:
Spinosyn A: 89 mg/L (89.4 in EFED and HED documents).
Spinosyn D: 0.495 mg/L
Working Note: 89.4 mg/L used as inputs for PRZM/EXAMS and GENEEC2 in EFED 2009a drinking water assessment.</td><td>Dow Elanco 1996;
Thompson et al. 2005
U.S. EPA/OPP/EFED 2011a
U.S. EPA/OPP/HED 2009a</td></tr>
<tr><td></td><td>Environmental Properties</td><td></td></tr>
<tr><td>Aquatic anaerobic metabolism, half-lives</td><td>Spinosyn A: 161 days
Spinosyn D: 250 days
Working Note: Spinosad assumed to be stable for PRZM/EXAMS and GENEEC2 in EFED 2009a,b drinking water assessment.</td><td>MRID 43507305, U.S. EPA/OPP/EFED 2005, 2009a,b
Also in Tomlin 2004</td></tr>
<tr><td></td><td>Spinosyn A: 160 days
Spinosyn D: 240 days</td><td>Cleveland et al. 2002a</td></tr>
<tr><td>Aqueous photolysis, half-lives</td><td>≈1 day, pH 7, 25°C</td><td>Dow Elanco 1996
Cleveland et al. 2002a</td></tr>
<tr><td>Bioconcentration in fish (BCF, L/kg)</td><td>Spinosyn A in rainbow trout, Maximum BCFs
Nonedible: 28.8 (at 28 days)
Edible: 7.5 (at 24 days)
Whole Fish: 21.1 (at 7 days)</td><td>MRID 43557601, U.S. EPA/OPP/EFED 2005, 2009a</td></tr>
<tr><td></td><td>Spinosyn D in rainbow trout, Maximum BCFs
Nonedible: 42 (at 11 days)
Edible: 20.5 (at 11 days)
Whole Fish: 41.9 (at 7 days)</td><td>MRID 44537734, U.S. EPA/OPP/EFED 2005, 2009a</td></tr>
<tr><td></td><td>Total Residues (Spinosyns A, D, and metabolites)
Nonedible: 103-152 (average = 127.5)
Edible: 16-47 (average = 31.5)
Whole Fish: 84-115 (average = 99.5)</td><td>U.S. EPA/OPP/EFED 2009a, p. 8
MRID not specified.</td></tr>
<tr><td>Field dissipation</td><td>Less than 2-3 weeks</td><td>Dow Elanco 1996</td></tr>
<tr><td>Foliar half-life</td><td>1.6-16 days</td><td>Tomlin 2004</td></tr>
<tr><td></td><td>35 days used as default …to account for the stability of spinosad (EFED p. 46).</td><td>U.S. EPA/OPP/EFED 2011a</td></tr>
<tr><td></td><td>Zucchini: 3.6 to 4.1 days [3.5, 3.6, 3.9, 3.9; Table 2 of paper]</td><td>Liu et al. 2013b</td></tr>
<tr><td></td><td>Cauliflower:
1.2 days at 15 g/ha
1.58 days at 30 g/ha
Average: 1.4 days</td><td>Mandal et al. 2009</td></tr>
<tr><td></td><td>Sweet pepper foliage
156 days with 60 mg/L solution
120 days with 120 mg/L solution
Working Note: Measured under greenhouse and not field conditions. As discussed by authors, much slower under greenhouse relative to field conditions.</td><td>Santis et al. 2012</td></tr>
</table>

<table>
<tr><th>Item</th><th>Value</th><th>Reference[1]</th></tr>
<tr><td></td><td>Half-lives in days<table><tr><th>Ap. Rate</th><th>Cabbage</th><th>Cauliflower</th></tr><tr><td>17.5 g/ha</td><td>1.5</td><td>2.8</td></tr><tr><td>35 g/ha</td><td>2.6</td><td>2.0</td></tr></table></td><td>Sharma et al. 2007</td></tr>
<tr><td></td><td>Chili Fruits
1.48 Days at 73 g/ha
6.72 days at 146 g/ha</td><td>Sharma et al. 2008</td></tr>
<tr><td></td><td>Cabbage
1.4 days at 15 g/ha
1.5 days at 30 g/ha</td><td>Singh and Battu 2012</td></tr>
<tr><td></td><td>Kiwi
Spinosyn A: 6.2, 6.1, 8, 8.2, 12 days
Spinosyn D: 10, 7.8, 10.4, 11, 16.5 days
Note: Increasing half-lives with increasing concentration of spinosyns.</td><td>Tomkins et al. 1991</td></tr>
<tr><td></td><td>Cowpea pods
1.05 - 1.39 days</td><td>Vijayasree et al. 2014</td></tr>
<tr><td></td><td>Egg Plant
Spinosyn A: 1.81 days
Spinosyn D: 1.61 days</td><td>Zhao et al. 2007</td></tr>
<tr><td>Hydrolysis</td><td>Spinosyn A: 200 days (pH 9)
Spinosyn D: 259 days (pH 9)
Both stable at pH5 and 7.</td><td>Dow Elanco 1996; Tomlin 2004; Cleveland et al. 2002a</td></tr>
<tr><td></td><td>Both Spinosyn A and D stable at 2 ppm solutions at 25±1 °C for 30 days.
Working Note: Spinosad assumed to be stable for PRZM/EXAMS and GENEEC2 in EFED 2009a,b drinking water assessment.</td><td>MRID 43507301, U.S. EPA/OPP/EFED 2005, 2009a,b</td></tr>
<tr><td>K_d K_{oc}</td><td>Spinosyn A<table><tr><th>Soil</th><th>K_d</th><th>K_{oc}</th></tr><tr><td>Sand</td><td>8.3</td><td>2,862</td></tr><tr><td>Loamy sand</td><td>5.4</td><td>831</td></tr><tr><td>Sandy loam</td><td>25</td><td>4,237</td></tr><tr><td>Silt loam</td><td>323</td><td>134,583</td></tr><tr><td>Clay Loam</td><td>283</td><td>21,938</td></tr></table>Working Note: Lowest non-sand Koc of 4,237 used for PRZM/EXAMS and GENEEC2 in EFED 2009a drinking water assessment.</td><td>MRID 43507306, U.S. EPA/OPP/EFED 2005</td></tr>
<tr><td></td><td>Spinosyn D<table><tr><th>Soil</th><th>K_d</th><th>K_{oc}</th></tr><tr><td>Sand</td><td>6.2</td><td>2,138</td></tr><tr><td>Loamy sand</td><td>4.3</td><td>622</td></tr><tr><td>Sandy loam</td><td>17</td><td>2,881</td></tr><tr><td>Silt loam</td><td>179</td><td>74,583</td></tr></table></td><td>MRID 43816602, U.S. EPA/OPP/EFED 2005</td></tr>
<tr><td>K_d</td><td>Spinosyn A:5.4-323
Spinosyn D: not determined.</td><td>Dow Elanco 1996; Tomlin 2004</td></tr>
<tr><td>Photolysis, surface</td><td>A few days (soil and plant surfaces)</td><td>Dow Elanco 1996</td></tr>
<tr><td>Photolysis, soil</td><td>Spinosyn A: 13.6 days in silt loam, natural sunlight, 25.0 ± 1.0°C, 30 day observation period.</td><td>MRID 44597733, U.S. EPA/OPP/EFED 2005</td></tr>
<tr><td></td><td>Spinosyn A: 74 day half-life
Spinosyn D: 41 day half-life
Applied to soil at 1015 g/ha, silt loam, natural sunlight, 25.0 ± 1.0°C, 30 day observation period.</td><td>MRID 43507303, U.S. EPA/OPP/EFED 2005</td></tr>
</table>

<table>
<tr><th>Item</th><th>Value</th><th>Reference[1]</th></tr>
<tr><td>Sediment half-life</td><td>Spinosyn A: 161 days
Spinosyn D: 250 days
Working Note: Spinosad assumed to be stable for PRZM/EXAMS and GENEEC2 in EFED 2009b drinking water assessment.</td><td>U.S. EPA/OPP/EFED 2009b</td></tr>
<tr><td>Soil half-life, aerobic</td><td>Spinosyn A: 9.4-17.3 days</td><td>Dow Elanco 1996</td></tr>
<tr><td></td><td>Spinosyn A: 28 (volcanic soil)
Spinosyn D: 37 (volcanic soil)</td><td>Cleveland et al. 2002a</td></tr>
<tr><td></td><td>Spinosyn A: 9 days (sandy loam), 17 days (silt loam)
Spinosyn D: 14 days (silt loam)
Much longer half-lives sterilized soils.</td><td>Hale and Portwood 1996</td></tr>
<tr><td></td><td>Spinosyn A: 9.4-17.3 days
Spinosyn D: 14.5 days</td><td>Tomlin 2004</td></tr>
<tr><td></td><td><table><tr><th>Soil</th><th>Spinosyn A ($t_{½}$ days)</th><th>Spinosyn D ($t_{½}$ days)</th></tr><tr><td>Silt loam</td><td>17.3</td><td>14.5</td></tr><tr><td>Sandy loam</td><td>9.4</td><td></td></tr></table>Working Note: Spinosad assumed to be stable for PRZM/EXAMS and GENEEC2 in EFED 2009a,b drinking water assessment.</td><td>MRID 43507304, U.S. EPA/OPP/EFED 2005, 2009a
Working Note: Identical to Hale and Portwood 1996 and Tomlin 2004.</td></tr>
<tr><td>Soil dissipation half-life</td><td>Spinosyn A
Silt loam: 0.5 days (Mississippi)
Loam: 0.3 days (California)</td><td>MRID 43714301, U.S. EPA/OPP/EFED 2005, 2009a</td></tr>
<tr><td></td><td>3.5 to 3.9 days [3.8, 4.1, 4.0, 3.6: Table 2 of paper]</td><td>Liu et al. 2013b</td></tr>
<tr><td></td><td>2.8 days (at 17.5 g/ha)
2.0 days (at 35 g/ha)</td><td>Sharma et al. 2007</td></tr>
<tr><td></td><td>Dissipation Halftimes
Spinosyn A
Forest litter: 11.7 days (exponential)
Soil under Forest canopy: 2 or 12.4 days (hyperbolic)
Spinosyn D
Within 7 days in soil and litter.</td><td>Thompson et al. 2002a,b</td></tr>
<tr><td></td><td>Spinosyn A: 1.87 days
Spinosyn D: 0.95 days</td><td>Zhao et al. 2007</td></tr>
<tr><td>Water Dissipation</td><td>Spinosad (A:D::85:15, 480 g/L formulation applied at 100 g/ha to outdoor tanks).
Half-lives
Parent: 1.5 days
Total Residues: 4 days</td><td>MRID 43848803, U.S. EPA/OPP/EFED 2005, 2009a</td></tr>
<tr><td></td><td>Outdoor microcosm half-life: 1.8 days (Spinosyns A and D)</td><td>Cleveland et al. 2002a.</td></tr>
</table>

[1] There a many sources of information on some standard values – e.g., molecular weight. In general, only two sources as cited for each value. More than two sources are cited only to highlight apparent discrepancies. Note: No data on spinosad is either USDA/ARS Pesticide Properties Database (http://www.ars.usda.gov/Main/docs.htm?docid=14199 or Knisel and Davis (2002).

See Section 2.2.2 for discussion.

Table 3: Representative Formulations of Spinosad Labelled for Forestry

Formulation, EPA Reg. No, Content[1]	Applications
Blackhawk®, 62719-523 Dispersible Granule, 36% a.i. (w/w), no inerts specified.	Listed Pests: 1.1 to 3.5 oz/acre [0.025 to 0.08 lb a.i./acre] Maximum Rate: 0.28 lb a.i./acre Maximum Applications: 3 with at least a 7 day interval. Maximum Seasonal Rate: 0.45 lb a.i./acre. Ground: At least 5 – 10 gal./acre. Fine to coarse droplets. Arial: At least 5gal/acre, 10 gal./acre for trees. Medium to fine droplets. Adjuvants: 0.25 to 0.5% (v/v) emulsified or methylated crop oil, organosilicones. No fuel or mineral oil. Field Solution: pH 6-9
Conserve SC® [2], 62719-291 Suspension concentrate, 11.6% (w/w), 1 lb a.i./gallon (Propylene glycol, 4.5%)	Arial: At least 5gal/acre, 10 gal./acre for trees. Medium to fine droplets. 3-5 applications per year with 7-10 day interval Rates for Tree Farms/Plantations: 4-16 oz/acre (0.03 – 0.125 lb a.i./acre). Maximum application rate for trees: 88 oz/acre (0.6875 lb a.i./acre) [3]. Maximum Seasonal Rate: 0.45 lb a.i./acre. Maximum number of applications: 6/year. Field Solution: pH 6-9
Entrust®, 62719-282, Wettable powder, 80% a.i. (w/w) (Kaolin 3.4%; Silica Ge.l 2%)	Ground: At least 5 – 10 gal./acre. Fine to coarse droplets. Arial: At least 5gal/acre, 10 gal./acre for trees. Medium to fine droplets. Maximum annual application rate: 0.45 lb a.i./acre. Maximum application rate for trees: 3 oz/acre (0.15 lb a.i./acre). Minimum application interval for trees: 6 days. Adjuvants: 0.25 to 0.5% (v/v), emulsified or methylated crop oil, organosilicones. No fuel or mineral oil. Maximum number of applications: 3-6/year depending on crop. Field Solution: pH 6-9
Entrust SC®, 62719-621, Soluble concentrate, 22.5% w/w, 2 lb a.i./gallon (Propylene glycol ≥12% ≤16%)	Ground: At least 5 – 10 gal./acre. Fine to coarse droplets. Arial: At least 5 gal/acre, 10 gal./acre for citrus trees. Medium to fine droplets. Adjuvants: 0.25 to 0.5% (v/v), emulsified or methylated crop oil, organosilicones. No fuel or mineral oil. Maximum application rate for trees: 10 oz/acre (0.078 lb a.i./acre). Maximum annual application rate: 0.45 lb a.i./acre. Minimum Treatment Interval: 6 days.
SpinTor 2SC®, 62719-294, Soluble concentrate, 22.8%, 2 lb a.i./gallon (Propylene glycol, NS)	Identical to Entrust SC.
Success®, 62719-292 Soluble concentrate, 22.8%, 2 lb a.i./gallon (Propylene glycol, 4%)	Identical to Entrust SC.

Source: Labels and SDSs from Greenbook (2015) with the exception of the MSDS for SpinTox 2SC® which is taken from www.MSDSonline.com. All formulations from Dow AgroSciences.

[1] Other ingredients as specified on SDSs.

[2] Labeled for aquatic applications with the following limitation: …*restricted to commercial facilities that utilize fully contained above or in-ground pools or containers for the purpose of commercial production of aquatic ornamental plants*.

[3] 88 oz ÷ 128 oz/gallon = 0.6875 gallons. 88 oz/acre = 0.6875 lb a.i./acre for a 1 lb a.i./gallon formulation. This is not consistent with the labelled maximum seasonal application rate of 0.45 lb a.i./acre.

Table 4: MSDS Mammalian Effects Summary of Selected Formulations

Formulation Name [1]	% a.i.	Rat Oral LD_{50} (mg/kg bw)	Rabbit Dermal LD_{50} (mg/kg bw)	Rat Inhalation LD_{50} (mg/L x 4 h)	Rabbit Skin Irritation	Rabbit Eye Irritation	Guinea Pig Skin Sensitization
Blackhawk® [DG]	36%	>5,000[3]	>5,000[3]	>5.51[3]	Non-irritating	Slight[8.9]	Negative
Conserve SC®	11.6%	>5,000[3]	>5,000[3]	>17.02 [3] dust/mist	Non-irritating	Slight[2, 9]	Negative
Entrust® [WP]	80%	>5,000[4]	>5,000[4]	>5.18[4]	Slight with redness	May cause [2, 7, 8, 9]	Negative[4]
Entrust SC®	22.5%	>5,000	>5,000	>4.19 aerosol	Non-irritating	Non-irritating [9]	Negative
SpinTor 2SC®	22.8%	>5,000[5]	>5,000	>5 aerosol	Slight with redness	Slight[2, 9]	Negative
Success® [SC]	22.8%	>5,000[3]	>5,000[3]	>5 aerosol [6]	Non-irritating	Slight[9]	Negative

Source: Material Safety Datasheets (MSDSs or SDSs) from www.greenbook.net or https://www.msdsonline.com.

[1]DG: Dispersible granule; SC: Suspension concentrate; WP: Wettable powder. Abbreviations in brackets [] are not part of the product name.
[2]*May cause pain disproportionate to the level of irritation to eye tissues.*
[3] Specified as "product" or formulation rather than active ingredient (spinosad). If value is not specified as active ingredient or formulation, no superscript is used.
[4] Specified as information on spinosad and not formulation. If value is not specified as active ingredient or formulation, no superscript is used.
[5] Specified for both rats and mice.
[6] *The LC_{50} has not been determined*. The value given is …*for similar material.*
[7] SDS states: *Causes serious eye irritation.*
[8] Product label states that the formulation may cause moderate eye irritation. Also stated on SDS for Blackhawk.
[9] MSDS/SDS recommends safety glasses.

See Table 3 a fuller description of the formulations.

Table 5: MSDS Summary of Ecological Effects for Selected Formulations

Formulation[1] Data	Blackhawk® [DG][6]	Conserve® SC	Entrust® [WP]	Entrust SC®	SpinTor 2SC®	Success® [SC]
% a.i.	36%	11.6%	80%	22.5%	22.8%	22.8%
Terrestrial Organisms[2]						
Birds (NOS) acute LD_{50}	>2000	>2000	>2000	>2000 [6]	>2000 [6]	>2000 [6]
Birds (NOS) acute LC_{50}	>5000	>5000		>5000 [6]	>5000 [6]	>5000 [6]
Quail, Acute LD_{50}	>2000	>2000	>2000	>2000 [6]		
Quail, Acute LC_{50}	>5253	>5253		>5253 [6]		
Mallard, Acute LD_{50}						
Mallard, Acute LC_{50}						
Honeybee oral LD_{50} [4]	0.06[9]	0.06[9]	0.49 [8]	0.06 [6,9]		
Honeybee contact LD_{50} [4]	0.05	0.05		0.05 [6]		
Earthworm LC_{50}	>970	>970		>970 [6]		
Aquatic Organisms[3]						
Fish, most sensitive Acute LC_{50}	0.1 to 1	0.1 to 1	0.1 to 1	0.1 to 1 [6]	0.1 to 1 [6]	0.1 to 1 [6]
Bluegill Acute LC_{50}	5.9	5.9		5.9 [6]		
Carp, Acute LC_{50}		4	>100 [7]			
Rainbow trout, Acute LC_{50}		27				
Rainbow trout, Chronic NOEC	0.5	0.5		0.5 [6]		
Daphnia Acute LC_{50}	1.5	1.5	1.5 [6]	1.5 [6]		
Daphnia Chronic NOEC	0.0012	0.0012		0.0012 [6]		
Oyster Acute LC_{50}	0.295	0.295	0.295 [6]	0.295 [6]		
Algae EC_{50}, *A. flos-aquae*		6.1				
Algae EC_{50}, *Navicula*	0.107	0.107	0.107 [6]	0.107 [6]		
Algae EC_{50}, *P. subcapitata*	39	39		39 [6]		
Lemna EC_{50}	10.6	10.6				

Source: Material Safety Datasheets (MSDSs) from www.greenbook.net or https://www.msdsonline.com.

[1]DG: Dispersible granule; SC: Suspension concentrate; WP: Wettable powder. Abbreviations in brackets [] are not part of the product name.

[2] All doses in mg/kg bw unless otherwise specified. All dietary concentrations in ppm (mg/kg diet) unless otherwise specified.

[3] All concentrations in mg/L.

[4] Dose in µg/bee.

[5] Concentration in soil in units of ppm (mg/kg soil).

[6] Specified as applicable to spinosad.

[7] Specified as applicable to formulation.

[8] This appears to be a typographical error. The correct value is probably 0.049 µg/bee (European Commission 2006). See Table 17 and Section 4.1.2.4.2 for discussion.

[8] The contact LC50 of 0.06 µg/bee is documented in Mayes et al. (2003). See Table 19 and Section 4.1.2.4.3 for discussion.

Table 6: Mammalian Sensitivities to Spinosad

Duration/Species [1]	NOAEL (mg a.i./kg bw/day)	LOAEL (mg a.i./kg bw/day)	Reference
Subchronic			
Mouse	7.5	22.5	MRID 43566602
Rat	36.4	73	MRID 43566601
Dog	5.1	10.1	MRID 43444102
Chronic			
Mouse	12.6	59	Bond et al. 1995a, MRID 43701505
Rat	10.8	27.3	Bond et al. 1995b, MRIDs 43701507 and 43710503
Dog	2.7	8.34	Harada 1995, MRID 43701504

[1] Reference Body Weights from Davies and Morris 1993: Mouse (0.02 kg), Rat (0.25 kg), Dog (10 kg).

[2] See Appendix 1, Table A1-2 for details. Separate LOAELs for male and female animals, when available, are averaged and rounded to the nearest 10^{th}.

See Section 3.1.5 for initial discussion.
See Figure 5 for illustration.

Table 7: Directed Foliar Applications - Derivation of Worker Exposure Rates

Item	Value	Reference/Note	Row
Reference Chemical	Glyphosate	Section 3.2.2.1.3.	2
First-order dermal absorption rate coefficient for reference chemical ($hour^{-1}$) [ka_{Ref}]	0.00041	SERA 2014b	3
Occupational Exposure Rates for Reference Chemical			4
Central Estimate	0.0003	SERA 2014b, Table 14	5
Lower 95% Prediction Bound	0.00006	SERA 2014b, Table 14	6
Upper 95% Prediction Bound	0.002	SERA 2014b, Table 14	7
Subject Chemical	Spinosyn A		8
First-order dermal absorption rate coefficient for subject chemical ($hour^{-1}$) [ka_P]	0.00002	Section 3.1.3.2.2	9
$ka_P \div ka_{Ref}$	0.0487804878		10
Occupational Exposure Rates for Subject Chemical (Imidacloprid)			11
Central Estimate	0.0000146341	SERA 2014b, Eq. 22	12
Lower 95% Prediction Bound	0.0000029268	SERA 2014b, Eq. 22	13
Upper 95% Prediction Bound	0.000097561	SERA 2014b, Eq. 22	14

See Section 3.2.1 for discussion.

Documentation for Table: The above table implements the adjustment of worker exposure rates based dermal absorption rates. The table uses MS Word "fields" rather than macros.

- Determine the first-order dermal absorption rate coefficient for the chemical under review. See SERA 2014a, Section 3.1.3.2.2.
- Select the reference chemical. See SERA 2014b, Section 4.1.6.1.
- Fill in the information on the reference chemical in the upper section of the above table.
- Fill in the first-order dermal absorption rate coefficient for the chemical under review in the Value column of Row 9 in the above table.
- Update the estimated values for ratio of the k_a values and the occupational exposure rates for the chemical under review – i.e., the green shaded cells in the above table. The simplest way to update these fields is to select each of the 4 green shaded cells (one at a time and in order), press the right mouse button, and select 'Update field'.

Table 8: Ground Broadcast Applications - Derivation of Worker Exposure Rates

Item	Value	Reference/Note	Row
Reference Chemical	2,4-D	Section 3.2.2.1.3.	2
First-order dermal absorption rate coefficient for reference chemical ($hour^{-1}$) [ka_{Ref}]	0.00066	SERA 2014b	3
Occupational Exposure Rates for Reference Chemical			4
Central Estimate	0.0001	SERA 2014b, Table 14	5
Lower 95% Prediction Bound	0.000002	SERA 2014b, Table 14	6
Upper 95% Prediction Bound	0.005	SERA 2014b, Table 14	7
Subject Chemical	Spinosyn A		8
First-order dermal absorption rate coefficient for subject chemical ($hour^{-1}$) [ka_P]	0.00002	Section 3.1.3.2.2	9
$ka_P \div ka_{Ref}$	0.0303030303		10
Occupational Exposure Rates for Subject Chemical (Imidacloprid)			11
Central Estimate	0.0000030303	SERA 2014b, Eq. 22	12
Lower 95% Prediction Bound	0.0000000606	SERA 2014b, Eq. 22	13
Upper 95% Prediction Bound	0.0001515152	SERA 2014b, Eq. 22	14

See Section 3.2.1 for discussion.

Documentation for Table: The above table implements the adjustment of worker exposure rates based dermal absorption rates. The table uses MS Word "fields" rather than macros.

- Determine the first-order dermal absorption rate coefficient for the chemical under review. See SERA 2014a, Section 3.1.3.2.2.
- Select the reference chemical. See SERA 2014b, Section 4.1.6.1.
- Fill in the information on the reference chemical in the upper section of the above table.
- Fill in the first-order dermal absorption rate coefficient for the chemical under review in the Value column of Row 9 in the above table.

Update the estimated values for ratio of the k_a values and the occupational exposure rates for the chemical under review – i.e., the green shaded cells in the above table. The simplest way to update these fields is to select each of the 4 green shaded cells (one at a time and in order), press the right mouse button, and select 'Update field'.

Table 9: Aerial Applications - Derivation of Worker Exposure Rates

Item	Value	Reference/Note	Row
Reference Chemical	2,4-D	Section 3.2.2.1.3.	2
First-order dermal absorption rate coefficient for reference chemical ($hour^{-1}$) [ka_{Ref}]	0.00066	SERA 2014b	3
Occupational Exposure Rates for Reference Chemical			4
Central Estimate	0.00002	SERA 2014b, Table 14	5
Lower 95% Prediction Bound	0.0000005	SERA 2014b, Table 14	6
Upper 95% Prediction Bound	0.0008	SERA 2014b, Table 14	7
Subject Chemical	Spinosyn A		8
First-order dermal absorption rate coefficient for subject chemical ($hour^{-1}$) [ka_P]	0.00002	Section 3.1.3.2.2	9
$ka_P \div ka_{Ref}$	0.0303030303		10
Occupational Exposure Rates for Subject Chemical (Imidacloprid)			11
Central Estimate	0.0000006061	SERA 2014b, Eq. 22	12
Lower 95% Prediction Bound	0.0000000152	SERA 2014b, Eq. 22	13
Upper 95% Prediction Bound	0.0000242424	SERA 2014b, Eq. 22	14

See Section 3.2.1 for discussion.

Documentation for Table: The above table implements the adjustment of worker exposure rates based dermal absorption rates. The table uses MS Word "fields" rather than macros.

Working Note: Triclopyr BEE is a factor of 2.38 more. 2,4-D is a factor of 1.96 less. Use 2,4-D.

- Determine the first-order dermal absorption rate coefficient for the chemical under review. See SERA 2014a, Section 3.1.3.2.2.
- Select the reference chemical. See SERA 2014b, Section 4.1.6.1.
- Fill in the information on the reference chemical in the upper section of the above table.
- Fill in the first-order dermal absorption rate coefficient for the chemical under review in the Value column of Row 9 in the above table.
- Update the estimated values for ratio of the k_a values and the occupational exposure rates for the chemical under review – i.e., the green shaded cells in the above table. The simplest way to update these fields is to select each of the 4 green shaded cells (one at a time and in order), press the right mouse button, and select 'Update field'.

Table 10: Precipitation, Temperature and Classifications for Standard Test Sites

Location	Precipitation	Temperature	Average Annual Rainfall (inches)	Average Annual Temperature (◦F)
HI, Hilo	Wet	Warm	126.06	73.68
WA, Quillayute [1]	Wet	Temperate	95.01	49.14
NH, Mt. Washington	Wet	Cool	98.49	27.12
FL, Key West	Average	Warm	37.68	77.81
IL, Springfield	Average	Temperate	34.09	52.79
MI, Sault Ste. Marie	Average	Cool	32.94	40.07
AR, Yuma Test Station	Dry	Warm	3.83	73.58
CA, Bishop	Dry	Temperate	5.34	56.02
AK, Barrow	Dry	Cool	4.49	11.81

[1] Based on composite estimation in WEPP using latitude 47.94 N and longitude -124.54 W.

Table 11: Input Parameters for Fields and Waterbodies Used in Gleams-Driver Modeling

Field Characteristics	Description	Pond Characteristics	Description
Type of site and surface (FOREST)	Field (0)	Surface area	1 acre
Treated and total field areas	10 acres	Drainage area:	10 acres
Field width	660 feet	Initial Depth	2 meters
Slope	0.1 (loam and clay) 0.05 (sand)	Minimum Depth	1 meter
Depth of root zone	36 inches	Maximum Depth	3 meters
Cover factor	0.15	Relative Sediment Depth	0.01
Type of clay	Mixed		
Surface cover	No surface depressions		

Stream Characteristics	Value
Width	2 meters
Flow Velocity	6900 meters/day
Initial Flow Rate	710,000 liters/day

GLEAMS Crop Cover Parameters[3]	Description	Value
ICROP	Weeds	78
CRPHTX	Maximum height in feet.	3
BEGGRO	Julian day for starting growth	32
ENDGRO	Julian day for ending growth	334

Application, Field, and Soil Specific Factors [1]	Code[3]	Clay	Loam	Sand
Percent clay (w/w/):	CLAY	50%	20%	5%
Percent silt (w/w/):	SILT	30%	35%	5%
Percent sand (w/w/):	N/A	20%	45%	90%
Percent Organic Matter:	OM	3.7%	2.9%	1.2%
Soil porosity (cc/cc):	POR	0.47	0.4	0.4
Soil erodibility factor (tons/acre):	KSOIL	0.24	0.3	0.02
SCS Runoff Curve Number [2]:	CN2	90	74	59
Evaporation constant (mm/d):	CONA	3.5	4.5	3.3
Saturated conductivity below root zone (in/hr):	RC	0.087	0.212	0.387
Saturated conductivity in root zone (in/hr)	SATK	0.087	0.212	0.387
Wilting point (cm/cm):	BR15	0.28	0.11	0.03
Field capacity (cm/cm):	FC	0.39	0.26	0.16

[1] The qualitative descriptors are those used in the QuickRun window of Gleams-Driver. Detailed input values for the soil types are given in the sub-table below which is adapted from SERA (2007b, Tables 2 and 3). All fields are run for about 6 months before the pesticide is applied in early summer.

[2] From Knisel and Davis (Table H-4), *Clay*: Group D, Dirt, upper bound; *Loam*: Group C, woods, fair condition, central estimate; *Sand*: Group A, meadow, good condition, central estimate.

[3]Codes used in documentation for GLEAMS (Knisel and Davis 2000) and Gleams-Driver (SERA 2007a)

Table 12: Chemical parameters used in Gleams-Driver modeling

Parameter	Values	Note/Reference
Halftimes (days)		
Aquatic Sediment	7,300	Note 1
Foliar	6 (1.5-35)	Note 2
Soil	7,300	Note 1
Water	7,300	Note 1
Soil $K_{o/c}$, mL/g	4,237 (831-134,583)	Note 3
Sediment K_d, mL/g	25 (5.4-323)	Note 3
Water Solubility, mg/L	89.4	Note 4
Foliar wash-off fraction	0.5	Note 5
Fraction applied to foliage	0.5	Standard assumption
Depth of Soil Incorporation	1 cm	Standard assumption
Irrigation after application	none	
Initial Application Date	June 15	Note 6

Notes

Number	Text
1	U.S. EPA/OPP/EFED 2009b assumes that spinosad is functionally stable to account for metabolites. GLEAMS does not accommodate an infinite half-life. The half-life of 20 years (7300 days) is functionally equivalent.
2	The central estimate is approximated from Sharma et al. (2008, high application rate) and lower bound values from Tomkins et al. (1991). The lower bound is taken from Vijayasree et al. (2014, cowpea). The upper bound is the default from U.S. EPA/OPP/EFED (2011a, p. 46). The upper bound may be an extreme worse-case scenario.
3	The values for K_{oc} and K_d are taken from MRID 43507306, the study used in the EPA drinking water assessment (U.S. EPA/OPP/EFED 2009b). EPA uses the lowest non-sand values, in this case the median value. For the current risk assessment, the central estimate is taken as the median value and the range is defined by the upper and lower bounds of values given in MRID 43507306. See Table 1 of the current risk assessment for details. These parameters are modeled using a triangular distribution.
4	Value for spinosyn A used by U.S. EPA/OPP/EFED (2009b) in drinking water assessment.
5	No data on foliar washoff has been identified. Default value used.
6	The application dates will be dependent on the pest species and local conditions (e.g., Lewis et al. 2007; Peusens and Belian 2012; Thompson et al. 2002a, b;). Mid-June is taken from the study by Lewis et al. 2007, an Forest Service/APHIS application for the control of EAB. A mid-June application was also used in a Forest Service efficacy study for the Nantucket pine tip moth (Nowak et al. 2000) and a Canadian forestry application (Thompson et al. 2002a).

Table 13: Summary of Modeled Concentrations in Surface Water

Scenario/Source	Peak Concentrations (ppb or µg/L per lb/acre)		Long-Term Average Concentrations (ppb or µg/L per lb/acre)	
Direct Spray and Spray Drift				
Pond, Direct Spray (Section 3.2.3.4.2) [1]	112		N/A	
Pond, drift at 25 feet (Section 3.2.3.4.2) [1]	25 (Aerial) 12 (High Ground boom) 3.9 (Low Ground boom) 0.93 (Backpack)		N/A	
Stream, Direct Spray (Section 3.2.3.4.2) [2]	91		N/A	
Stream, drift at 25 feet (Section 3.2.3.4.2) [2]	20 (Aerial) 9.5 (High Ground boom) 3.2 (Low Ground boom) 0.76 (Backpack)		N/A	
One Application (Appendix 8)				
Pond, Section 3.2.3.4.4	**Soil**	**Conc.**	**Soil**	**Conc.**
	Clay	18.7 (1.6 - 172)	Clay	6.05 (0.5 - 67)
	Loam	7.26 (0.06 - 60)	Loam	2.37 (0.005 - 22.5)
	Sand	0.62 (0 - 16.2)	Sand	0.183 (0 - 6.6)
Stream, Section 3.2.3.4.4,	**Soil**	**Conc.**	**Soil**	**Conc.**
	Clay	10.8 (1.95 - 106)	Clay	0.33 (0.02 - 2.24)
	Loam	7.51 (0.15 - 146)	Loam	0.142 (0.0009 - 1.14)
	Sand	1.55 (0 - 63)	Sand	0.0161 (0 - 0.4)
Two Applications, 6 day interval (Appendix 9)				
Pond, Section 3.2.3.4.4	**Soil**	**Conc.**	**Soil**	**Conc.**
	Clay	37.1 (3.2 - 340)	Clay	12.1 (1 - 134)
	Loam	14.4 (0.12 - 120)	Loam	4.71 (0.01 - 45)
	Sand	1.25 (0 - 33)	Sand	0.37 (0 - 13.3)
Stream, Section 3.2.3.4.4	**Soil**	**Conc.**	**Soil**	**Conc.**
	Clay	21.6 (3.9 - 213)	Clay	0.65 (0.04 - 4.5)
	Loam	14.8 (0.31 - 294)	Loam	0.294 (0.0018 - 2.28)
	Sand	3.09 (0 - 128)	Sand	0.032 (0 - 0.9)
EPA Tier 1 Models (Appendix 10)				
FIRST (Reservoir model)				
Single Application	23 (17-43.4)		6.5 (0.52-11)	
Two Applications	46 (33.9-86.8)		13.1 (1-22)	
PRZM-GW (Ground water)				
Single Application	0.03 (0.006-0.7)		N/A	
Two Applications	0.06 (0.012-1.4)		N/A	
EPA PRZM/EXAMS Tier 2[3]				
Bulb Vegetables	4.6		2.4	

[1] See Attachment 1, Worksheet B04c. Values normalized by dividing by the application rate of 0.225 lb a.i./acre and converting from mg/L to µg/L.

[2] See Attachment 1, Worksheet B04d. Values normalized by dividing by the application rate of 0.225 lb a.i./acre and converting from mg/L to µg/L.

[3] Data from U.S. EPA/OPP/EFED (2005), p. 33. Maximum acute modelled concentration of 2.15 µg/L for 5 applications at an application rate of 0.094 lb a.i./acre. WCR = 2.15 µg/L ÷ (0.094 x 5) ≈ 4.5745 µg/L per lb a.i./acre. 60-day concentration of 1.12 µg/L for 5 applications at an application rate of 0.094 lb a.i./acre. WCR = 1.12 µg/L ÷ (0.094 x 5) ≈ 2.383 µg/L per lb a.i./acre.

Table 14: Concentrations in surface water used in this risk assessment

Foliar Broadcast, one application	**Peak WCR[1]**	**Longer-term WCR[1]**
Central[2]	0.0073	0.0025
Lower[3]	0.00008	0.000015
Upper[4]	0.17	0.067
Foliar Broadcast, two applications	**Peak WCR[1]**	**Longer-term WCR[1]**
Central[2]	0.014	0.005
Lower[3]	0.00012	0.000027
Upper[4]	0.34	0.13

[1] WCR (Water contamination rates) – concentrations in units of mg a.i./L expected at an application rate of 1 lb a.i./acre. Units of mg a.i./L are used in the EXCEL workbook that accompanies this risk assessment. All values rounded to two significant digits.

[2] The central estimates are based on GLEAMS-Driver simulations for loam soils using central estimates for a pond. See Table 13 for details.

[3] The lower bound estimates are based on the lower bound estimate for a pond in in areas with sandy soils, high rainfall and low temperatures. Lower (essentially zero) concentrations may occur in areas with moderate to low rainfall. See Appendices 7 and 8, Tables 6 and 7, for details.

[4] The upper bound estimates are based on GLEAMS-Driver simulations for clay soils using upper bound values for a pond. See Table 13 for details.

See Section 3.2.3.4.6 for discussion.

Table 15: Estimated residues in food items per lb a.i. applied

Food Item	Central [a]	Lower [b]	Upper [a]
Standard Values			
Short grass	85	30	240
Tall grass	36	12	110
Broadleaf/forage plants and small insects	45	15	135
Fruits, pods, seeds, and large insects	7	3.2	15
Values for Spinosad			
Cauliflower (Mandel et al. 2009) [1]	14.2 16.7		
Cowpea pods (Vijayasree et al. 2014) [2]	14.4 14.6		

All concentration given in units of ppm (mg agent/kg food) per lb a.i./acre.

[a] U.S. EPA/EFED 2001, p. 44 as adopted from Fletcher et al. (1997).
[b] Central values × (Central Value ÷ Upper Value).

[1] Three applications at 15 g a.i./ha [0.0134 lb a.i./acre] yielded initial residues of 0.57 mg/kg.
Residue rate calculated as: 42.5 ppm/lb/acre [$0.57 \div 0.0134 \approx 42.5$; $42.5 \div 3 \approx 14.2$]
Three applications at 30 g a.i./ha [0.0268 lb a.i./acre] yielded initial residues of 1.34 mg/kg.
Residue rate calculated as: 42.5 ppm/lb/acre [$1.34 \div 0.0268 \approx 50$; $50 \div 3 \approx 16.7$]

[2] One application at 73 g a.i./ha [0.0651 lb a.i./acre] yielded initial residues of 0.94 mg/kg.
Residue rate calculated as: 14.4 ppm/lb/acre [$0.94 \div 0.0651 \approx 14.4393$]
One application at 146 g a.i./ha [0.1302 lb a.i./acre] yielded initial residues of 1.9 mg/kg.
Residue rate calculated as: 14.6 ppm/lb/acre [$1.9 \div 0.1202 \approx 14.56$]

Table 16: Summary of toxicity values used in human health risk assessment

Acute – short-term incidental (1-30 days)

Element	Derivation of RfD
EPA Document	U.S. EPA/OPP/HED 2011a, Table A.2.1.
Study	MRID 43444102, 13-week subchronic, spinosad
NOAEL Dose	4.9 mg/kg bw/day
LOAEL Dose	9.73 mg/kg bw/day
LOAEL Endpoint(s)	Pathologies in several organs, decreased body weights, anemia, possible liver damage.
Species, sex	Dogs, males and females
Uncertainty Factor/MOE	100
Equivalent RfD	0.049 mg/kg bw/day

Note: The EPA risk assessments use these values for short-term (1-30 days) dermal and inhalation exposures.

See Section 3.3.2 for discussion of acute toxicity value.

Chronic – lifetime exposure

Element	Derivation of RfD
EPA Document	U.S. EPA/OPP/HED 2011a, Table A.2.1.
Study	MRID 47011901 [Spinetoram]
NOAEL Dose	2.49 mg/kg bw/day
LOAEL Dose	5.36 mg/kg bw/day
LOAEL Endpoint(s)	Pathologies in several organs of males and females.
Species, sex	Dogs, males and females
Uncertainty Factor	100
Chronic RfD	0.0249

See Section 3.3.3 for discussion of chronic toxicity value.

Table 17: Oral or Injection LD_{50} Values in Terrestrial Invertebrates

Species	Hrs	Form[1]	LD_{50} (ng)	BW[2] (mg)	LD_{50} (mg/kg bw)[4]	Reference
Bees [Hymenoptera: Apidae][2]						
Apis mellifera	48	TGAI	47.11	116	0.41	Carvalho et al. 2013
Apis mellifera	N.S.	TGAI	57	116	0.49	European Commission 2006
Apis mellifera	48	TGAI	63	128	0.492	Mayer et al. 2001
Apis mellifera	24	TGAI	60	116	0.52	Miles et al. 2002
Apis mellifera	48	TGAI	53	116	0.50	Mayes et al. 2003
Apis mellifera	N.S.	NAF-85	49	116	0.42	European Commission 2006
Apis mellifera	48	480 SC	53	116	0.50	Mayes et al. 2003
Bombus terrestris	48	480 SC	38.5	300	0.13	Mayes et al. 2003
Melipona quadrifasciata	24	480 SC	12.07	80	0.15	Tom et al. 2015
Blattodea: Blattidae						
Periplaneta americana[3]	24	Spyn A	740	700	1.1	Salgado 1998
Periplaneta americana[3]	24	Spyn A	1900	700	2.7	Salgado 1998
Lepidoptera						
Heliothis virescens (larvae) [3]	24	Spyn A	14	60	0.23	Salgado 1998

[1] TGAI: Technical grade; Spyn A: spinosyn A; NAF-85: 44.2% formulation (Tracer); 240 SC: 240 g a.i./L SC formulation (NOS); 480 SC: 480 g a.i./L SC formulation (NOS),

[2] Excludes reported LD_{50} of 0.06 mg a.i./bee [60 µg or 60,000 ng/bee] from Cleveland et al. 2002b, Table 5. See text for discussion.

[3] Injection exposure.

[4] The average body weight of 128 mg is reported in the study by Mayer et al. 2001.

[5] ng/mg = µg/g = mg/kg

See Section 4.1.2.4.2 for discussion.
See Appendix 3 for details.
See Table 18 for insect body weights used to estimate doses in units of mg/kg bw.

Table 18: Reference Body Weights used for insects

Species	Body Weight (mg)	Reference
Aedes aegypti	2.85	Pridgeon et al. 2008, Table 2
Anopheles quadrimaculatus	1.92	Pridgeon et al. 2008, Table 4
Apis mellifera	116	Winston (1987, p. 54)
Bombus impatiens	150	Franklin et al. (2004)
Bombus terrestris	300	Thompson 2015, Table 1, p. 2
Bactrocera dorsalis	15	Lin et al. 2013, Table 2, p. 356
Bactrocera cucurbitae	15	Use value for *Bactrocera dorsalis*
Culex quinquefasciatus	2.02	Pridgeon et al. 2008, Table 3
Helicoverpa armigeram	11.5	Hertog et al. 2002, Table 5, average of range 8-15 mg.
Heliothis virescens (larva)	60	Salgado 1998, p. 95, 50 to 70 mg
Hyposoter didymator (pupa)	19.8	Schneider et al. 2003
Manduca sexta	30	Hertog et al. 2002, average of 20 to 40 mg range
Musca domestica	30	Zanuncio et al. 2005, Figure 1a, p. 774
Megachile rotundata	30	Thompson 2015, Table 1, p. 2 and Meyer et al. 2001
Melipona quadrifasciata	80	Thompson 2015, Table 1, p. 2, average of 2 other *Melipona* species.
Nomia melander	85	Mayer et al. 2001
Periplaneta americana	700	Wharton et al. 1965, Figure 1 and Table 6 (fed animals)

See Section 4.1.2.4.2 for initial discussion.
Note: These data are also used for contact toxicity studies (i.e., Table 19).

Table 19: Contact LD_{50} Values in Terrestrial Invertebrates

Species	Hrs	Form[1]	LD_{50} (ng)	BW[2] (mg)	LD_{50} (mg/kg bw)	Reference[3]
Hymenoptera: Apidae & others [3]						
Apis mellifera	48	TGAI	2.9	116	0.025	Hoxter et al. 1992 [2]
Apis mellifera	N.S.	N.S.	3.6	116	0.031	European Commission 2006
Apis mellifera	48	TGAI	40	116	0.34	Mayes et al. 2003[5]
Apis mellifera	48	TGAI	47.11	116	0.41	Carvalho et al. 2013
Apis mellifera	24	1.6% WP	50	116	0.43	Miles et al. 2002
Apis mellifera[4]	24	TGAI	78	127	0.61	Mayer et al. 2001
Apis mellifera	48	480 SC	60	116	0.52	Mayes et al. 2003[5]
Apis mellifera	24	480 SC	880	116	7.6	Miles 2003
Apis mellifera	48	480 SC	900	116	8.5	Mayes et al. 2003[5]
Megachile rotundata [Megachilidae] [4]	24	TGAI	58	30	1.9	Mayer et al. 2001
Nomia melander [Halictidae] [4]	24	TGAI	65	85	0.76	Mayer et al. 2001
Bombus terrestris	48	480 SC	19,400	300	65	Mayes et al. 2003[5,6]
Hymenoptera: Ichneumonidae						
Hyposoter didymator[p]	48	SC	N.S.	19.8	0.5	Schneider et al. 2003
Diptera: Muscidae						
Musca domestica	72	N.S.	24.2	30	0.8	Scott 1998
Musca domestica	72	TGAI	0.74	30	0.025	Shi et al. 2011
Diptera: Culicidae						
Aedes aegypti	24	N.S.	N.S.	2.85	0.89	Pridgeon et al. 2008
Culex quinquefasciatus	24	N.S.	N.S.	2.02	2.02	Pridgeon et al. 2008
Anopheles quadrimaculatus	24	N.S.	N.S.	1.92	1.5	Pridgeon et al. 2008
Diptera: Tephritidae fruit fly						
Bactrocera dorsalis	24	TGAI	59.6	15	3.97	Hsu and Fend 2006
Bactrocera dorsalis	24	TGAI	40.9	15	2.73	Hsu et al. 2012b
Bactrocera cucurbitae	24	SC	5.0	15*	0.33	Hsu et al. 2012a
Lepidoptera: Sphingidae						
Manduca sexta (larvae)	48	N.S.	2.0	30	0.067	Herzog et al. 2002
Lepidoptera: Noctuidae						
Helicoverpa armigeram	48	N.S	N.S.	11.5	1.6	Achaleke et al. 2009

[1] TGAI: Technical grade; N.S. Not specified; SC formulation; 240 SC: 240 g a.i./L SC formulation (NOS); 480 SC: 480 g a.i./L SC formulation (NOS)
[p]=pupa

[2] MRID 43414547. A very similar LD_{50} of 2.5 µg/bee is reported in review by Miles (2003). This is probably identical to Hoxter study.

[3] Reported LD_{50} values of 0.0025 mg a.i./bee and 0.045 mg/bee from Cleveland et al. 2002b are excluded. The units appear to be an error.

[4] Average body weights for each species reported in paper. See Appendix 3 for confidence intervals, which overlap for all three species.

[5] Mayes et al.(2003) is an open literature review summarizing many unpublished studies from Dow. See Appendix 3 for details and citations to unpublished studies.

[6] This atypical value is attributed by Mayes et al. 2003 to an unpublished study by Aldershof l999a. This study is not summarized in EPA's ECOTOX database (https://cfpub.epa.gov/ecotox/) or other EPA documents.

See Appendix 3 for details.
See Section 4.1.2.4.3 for discussion.
For insect body weights, see Table 18.
See Figures 6 and 7 for illustration.

Table 20: Relative toxicity to bees following direct spray

Species [Family]	48-hour LC_{50} (mg/L)	Confidence Interval on LC_{50} (mg/L)	Sensitivity Relative to the Honeybee [1]	Reference
Honey bee, *Apis mellifera* [Apidae]	22	18-25	1	Bailey et al. 2005
Alfalfa leafcutting bee, *Megachile rotundata* [Megachilidae]	12.5	11.3-14	0.56	Scott-Dupree et al. 2009
Bumblebee, *Bombus impatiens* [Apidae]	89.5	79.2-100.6	4.07	Scott-Dupree et al. 2009
Blue orchard bee, *Osmia lignaria* [Megachilidae]	47.0	40-54	2.14	Scott-Dupree et al. 2009

[1] LC50 for other species ÷ LC50 for honeybee.

See Section 4.1.2.4.3 for discussion.
See Appendix 3 for details.

Table 21: IOBC Summary Scores for Spinosad

Reference Arthropod Group	1	2	3	4	Total	Weighted Score
Williams et al. 2003b [1]						
Hemiptera	8	2	1	0	11	1.36
Neuroptera	22	2	1	2	27	1.37
Coleoptera	12	0	0	2	14	1.43
Dermaptera	0	0	3	0	3	3.00
Hymenoptera, Wasps	5	5	10	25	45	3.22
Acari	0	1	0	3	4	3.50
Miles and Eelen 2006 [2]						
Coleoptera, Coccinellidae	26	1	0	1	28	1.14
Neuroptera	22	3	1	0	26	1.19
Araneida	11	2	1	0	14	1.29
Hymenoptera, Ants	3	2	0	0	5	1.40
Hemiptera	60	9	3	7	79	1.46
Acari	40	3	1	6	40	1.46
Diptera	5	0	0	1	6	1.50
Coleoptera, Other	11	2	1	2	16	1.63
Thysanoptera	0	1	1	0	2	2.50
Hymenoptera, Wasps	13	14	13	43	83	3.04

[1] See Williams et al. 2003b, Table 2, Laboratory studies, n=104.
[2] See Miles and Eelen 2006, Table 3, laboratory, semi-field, and field studies, n=299.

See Figure 8 for illustration.
See Section 4.1.2.4.4.1 for discussion.

Table 22: Summary of Field/Field Simulation Studies with Bees

Type of Study/Organism	Application Rate (lb a.i./acre)	Observation	Reference to Appendix 3, Table A3-4 [1]
Field Simulation, Bumblebees	0.07 [2]	No impact on colony health but lower worker larval weights and possibly impaired foraging with signs of trembling.	Morandin et al. 2005
Field Simulation and Field Studies, Honeybees and Bumblebees	≤0.16	No effects.	Several studies summarized in Mayes et al. 2003 as well as Burns et al. 2001.
Field, Honeybee hives	0.19	Increase mortality (not statistically significant). No effect on foraging.	Mayes et al. 2003 [Kirkland 1999]
Field Simulation, Honeybees	0.2	Transient effect on foraging, no reduction in brood development.	Mayes et al. 2003 [Halsall 2002]
Field Simulation, Honeybees	0.48	Increase mortality on DAT 1 (not statistically significant) with reduction in brood development.	Mayes et al. 2003 [Vinall 2000]
Field Simulation, Bumblebees	0.71 [2]	Impaired colony health. Declines in number of workers, weights of workers, and increased mortality.	Morandin et al. 2005

[1] Mayes et al. (2003) summarize a large number of unpublished studies which are explicitly designated. The unpublished studies cited by Mayes et al. 2003 are given in brackets. See Appendix 3, Table A3-4 for details.

[2] Approximated application rate associated with residues of spinosad in pollen.

See Appendix 3, Table A3-4 for details.
See Section 4.1.2.4.4.2 for discussion.

Table 23: Summary of LOAEC from Field Studies

Order	Family	Species, Scientific Name	Species, Common Name	lb/acre	Relative Frequency within Order
Coleoptera	Chrysomelidae	*Zygogramma exclamationis*	Sunflower Beetle	0.045	0.13
Coleoptera	Chrysomelidae	*Leptinotarsa decemlineata*	Colorado Potato Beetle	0.045	0.38
Coleoptera	Chrysomelidae	*Epitrix fuscula*	Eggplant Flea Beetle	0.045	0.63
Coleoptera	Chrysomelidae	*Leptinotarsa decemlineata*	Colorado Potato Beetle	0.053	0.88
Diptera	Agromyzidae	*Liriomyza trifolii*	Serpentine Leafminer	0.090	0.50
Hemiptera	Anthocoridae	*Orius insidiosus*	Minute Pirate Bug	0.089	0.17
Hemiptera	Anthocoridae	*Orius insidiosus*	Minute Pirate Bug	0.090	0.50
Hemiptera	Anthocoridae	*Orius insidiosus*	Minute Pirate Bug	0.199	0.83
Lepidoptera	Noctuidae	*Chrysodeixis includens*	Soybean Looper Moth	0.016	0.02
Lepidoptera	Noctuidae	*Chrysodeixis includens*	Soybean Looper Moth	0.025	0.05
Lepidoptera	Noctuidae	*Trichoplusia ni*	Cabbage Looper	0.026	0.09
Lepidoptera	Pieridae	*Pieris rapae*	Cabbage White	0.026	0.13
Lepidoptera	Plutellidae	*Plutella xylostella*	Diamondback Moth	0.026	0.16
Lepidoptera	Noctuidae	*Chrysodeixis includens*	Soybean Looper Moth	0.030	0.20
Lepidoptera	Noctuidae	*Anticarsia gemmatalis*	Velvetbean Caterpillar	0.030	0.23
Lepidoptera	Noctuidae	*Trichoplusia ni*	Cabbage Looper	0.045	0.27
Lepidoptera	Pieridae	*Pieris rapae*	Cabbage White	0.045	0.30
Lepidoptera	Plutellidae	*Plutella xylostella*	Diamondback Moth	0.045	0.34
Lepidoptera	Noctuidae	*Spodoptera exigua*	Beet Armyworm	0.045	0.38
Lepidoptera	Noctuidae	*Helicoverpa zea*	Corn Earworm	0.045	0.41
Lepidoptera	Sphingidae	*Manduca sexta*	Hawk Moth	0.045	0.45
Lepidoptera	Noctuidae	*Chrysodeixis includens*	Soybean Looper Moth	0.045	0.48
Lepidoptera	Noctuidae	*Spodoptera exigua*	Beet Armyworm	0.050	0.52
Lepidoptera	Sphingidae	*Manduca sexta*	Hawk Moth	0.050	0.55
Lepidoptera	Noctuidae	*Chrysodeixis includens*	Soybean Looper Moth	0.050	0.59
Lepidoptera	Noctuidae	*Spodoptera exigua*	Beet Armyworm	0.060	0.63
Lepidoptera	Noctuidae	*Chrysodeixis includens*	Soybean Looper Moth	0.060	0.66
Lepidoptera	Noctuidae	*Spodoptera exigua*	Beet Armyworm	0.067	0.70
Lepidoptera	Noctuidae	*Chrysodeixis includens*	Soybean Looper Moth	0.067	0.73
Lepidoptera	Crambidae	*Ostrinia nubilalis*	European Corn Borer	0.068	0.77
Lepidoptera	Noctuidae	*Spodoptera exigua*	Beet Armyworm	0.073	0.80
Lepidoptera	Gelechiidae	*Anarsia lineatella*	Peach Twig Borer	0.094	0.84
Lepidoptera	Tortricidae	*Rhyacionia frustrana*	Nantucket Pinetip Moth	0.098	0.88
Lepidoptera	Noctuidae	*Spodoptera frugiperda*	Fall Armyworm	0.100	0.91
Lepidoptera	Noctuidae	*Spodoptera exigua*	Beet Armyworm	0.101	0.95
Lepidoptera	Noctuidae	*Agrotis ipsilon*	Cutworm	0.269	0.98
Orthoptera	Acrididae	*Melanoplus femurrubrum*	Redlegged Grasshopper	0.090	0.50
Hymenoptera	Pteromalidae	*Catolaccus grandis*	Ectoparasitoid Wasp	0.022	0.10
Hymenoptera	Chalcidoidea	*Trichogramma exiguum*	Parasitic Wasp	0.070	0.30
Hymenoptera	Eurytomidae	*Eurytoma pini*	Chalcid Wasp	0.098	0.50
Hymenoptera	Chalcididae	*Haltichella rhyacioniae*	Chalcid Wasp	0.098	0.70
Hymenoptera	Braconidae	*Macrocentrus ancylivorus*	Parasitic Wasp	0.098	0.90

Data from EXOTOX (2016).
See Figure 9 for illustration.
See Section 4.1.2.4.5 for discussion.

Table 24: Resistance and Variability to Spinosad in Terrestrial Insects

Orders Species	Resistance Factors	Comment [1]	Reference
Coleoptera			
Cryptolestes ferrugineus	1.7	Field versus laboratory populations. No additional resistance pressure. Variability rather than resistance?	Huang et al. 2004
Rhyzopertha dominica	0.6-1.0	Field versus laboratory populations. No additional resistance pressure. Variability rather than resistance?	Huang et al. 2004
Tribolium castaneum	4.8-7.5	Field versus laboratory populations. No additional resistance pressure. Variability rather than resistance?	Huang et al. 2004
Diptera			
Bactrocera cucurbitae	≈10 to 13.3	Field populations. No additional selection pressure.	Hsu et al. 2012a
Bactrocera dorsalis	**>480**	**Eight generations of resistance pressure based on survival of bioassays at LD_{70}. No marked cross-resistance to other pesticides.**	**Hsu and Feng 2006**
Bactrocera dorsalis	**>2445**	**30 generations of selection pressure. Mechanism unclear but possibly related to changes in receptor.**	**Hsu et al. 2012b**
Musca domestica	>150	Field population. No additional resistance pressure. Resistance apparently due to altered target site	Gao et al. 2007a
Drosophila melanogaster	3.8 to 7408	Various resistant and sensitive strains. No selection pressure during study.	Rinkevich and Scott 2013
Musca domestica	**21**	**Selection pressure (variable dosing) over 22 generations. Potential partial involvement of P450.**	**Markussen and Kristensen 2012**
Musca domestica	0.9 to 4.3	Strains with known resistance to other pesticides. No additional selection pressure.	Scott 1998
Musca domestica	**279**	**Selection pressure (60-80% lethal doses) over 27 generations. No apparent involvement of P450.**	**Shi et al. 2011**
Lepidoptera			
Cnaphalocrocis medinalis	0.39-3	Field populations subject to three years of spinosad field use. No other substantial selection pressure.	Zhang et al. 2014
Helicoverpa armigera	0.5 to 1.6	No cross resistance to spinosad in a strain resistant to pyrethroids.	Achaleke et al. 2009
Plodia interpunctella	1.75	Field versus laboratory populations. No additional resistance pressure. Variability rather than resistance?	Huang et al. 2004
Plutella xylostella	**1983**	**Selection pressure for 11 generation. Resistance associated with increased P450 activity.**	**Sayyed et al. 2008**
Tuta absoluta	**3150**	**Resistance factor based on survivors of acute bioassays over 22 generations. Resistance rapidly lost after removal of selection pressure. No apparent relationship of resistance to P450 activity.**	**Campos et al. 2014**
Thysanoptera			
Frankliniella occidentalis	**3,682 to 2,968,500**	**15 wild caught strains in areas with spinosad use. Resistance pressure for at least 7 generations (LD_{50}s). 1-19 range of variability in sensitive strains. No apparent relationship of resistance to P450. No cross resistance with other pesticides.**	**Bielza et al. 2007**
Frankliniella occidentalis	**20,000 to >100,000**	**Resistance pressure based on survivors of LD_{50} exposures over 4 generations. Resistance stable for up to 8 months in the absence of breeding with sensitive strains.**	**Bielza et al. 2008**
Frankliniella occidentalis	**170,000**	**Field resistant population with 3 additional generations of selection pressure. Based on cross breeding, resistance is a recessive trait.**	**Hou et al. 2014**
Thrips palmi	834.9 to 1957.5	Wild caught resistant strains. No additional resistance pressure. Resistance associated with cytochrome P450-mediated detoxification and receptor site insensitivity.	Bao et al. 2014

[1] Studies in bold type use artificial selection pressure to generate resistance.

See Section 4.1.2.4.6 for discussion.

Table 25: Overview of Acute Lethality Studies in Aquatic Invertebrates

Species	Agent	Hours	EC_{50} (mg a.i./L)	Reference[1]
Daphnids				
Daphnia magna	TGAI	48	7.37	Cleveland et al. 2002b
Daphnia magna	TGAI	48	14	Milazzo et al. 1994, MRID 43574502
Daphnia magna	TGAI	48	9.1	European Commission 2006
Daphnia magna	Success® formulation	48	0.0048	Deardorff and Stark 2009
Daphnia pulex	Success® formulation	48	0.129	Deardorff and Stark 2009
Ceriodaphnia dubia	Success® formulation	48	0.0018	Deardorff and Stark 2009
Mosquito Larvae				
Aedes aegypti	TGAI	24	0.35	Darriet et al. 2005
Aedes aegypti , 3rd instar	Laser®	48	0.007	Romi et al. 2006
Aedes aegypti, 4th instar	Tracer® Naturalyte	1	0.026	Perez et al. 2007
Aedes aegypti	N.S.	24	51.7	Kovendan et al. 2012
Aedes albopictus, 4th instar	TGAI, 88%	24	0.3	Liu et al. 2004b
Aedes albopictus, 4th Instar	Tracer® 24SC	48	0.019	Khan et al. 2011
Anopheles gambiae	TGAI	24	0.01	Darriet et al. 2005
Anopheles stephensi, 1st instar	NOS	24	0.002	Kumar et al. 2011
Anopheles stephensi, 3rd instar	Laser®	48	0.024	Romi et al. 2006
Culex pipiens, 3rd instar	Laser®	48	0.0032	Romi et al. 2006
Culex pipiens, 3rd instar	Conserve®	24	0.027	Cetin et al. 2005
Culex pipiens, 4th instar	Tracer® 12% SC	24	0.087	Mansour et al. 2012
Culex quinquefasciatus	TGAI	24	0.093	Darriet et al. 2005
Culex quinquefasciatus, 2nd instar	TGAI	48	0.019	Jiang and Mulla 2009
Culex quinquefasciatus, 4th instar	TGAI	48	0.026	Jiang and Mulla 2009
Culex quinquefasciatus, 4th instar	TGAI, 88%	24	0.1	Liu et al. 2004a
Culex quinquefasciatus, 3rd instar	Natular® XRG	24	0.196 to 0.490	Su and Chen 2014b
Culex quinquefasciatus, 2nd instar	11.6% formulation	48	0.01	Jiang and Mulla 2009
Culex quinquefasciatus, 4th instar	11.6% a.i. formulation	48	0.013	Jiang and Mulla 2009
Culex quinquefasciatus, 3rd instar	Natular® 2EC	72	0.031	Jones and Ottea 2013
Other				
Chironomus circumdatus (midge)	NOS	24	0.009	Kumar et al. 2011
Crassostrea virginica (bivalve)	TGAI	96	0.3	MRID 43571203
Palaemonetes pugio (shrimp)	TGAI	96	>9.76	MRID 43414539
Leptocheirus plumulosus (scud)	TGAI	240	1.38	MRID 47702901

[1] Does not include data on resistant species. See Section 4.3.3.3.1.2 for discussion.

See Appendix 6, Table A6-1 for details.
See Section 4.1.3.3.1 for discussion.

Table 26: Overview of Chronic Studies on the Toxicity of Spinosad to Aquatic Invertebrates

Group [Order: Family] Species	Agent	Days	NOAEC (µg a.i./L)	LOAEC (µg a.i./L)	Reference
Daphnids [Cladocera: Daphniidae]					
Daphnia magna	TGAI, 88%	21	0.62	1.2	MRID 43848801[1,2]
Daphnia magna	NOS	21	1.2	N.S.	European Commission 2006[2]
Daphnia magna	NOS	21	8	N.S.	European Commission 2006[3]
Daphnia magna	*Conserve® 120SC*	14	N.D.	8	Duchet et al. 2010b
Daphnia pulex	*Conserve® 120SC*	14	N.D.	8	Duchet et al. 2010b
Daphnia pulex	Success® formulation	60[4]	N.D.	2	Stark and Vargas 2003
Daphnia pulex	NOS	10		129 [5]	Stark 2005
Ceriodaphnia dubia	Success® formulation	8	0.5	1	Deardorff and Stark 2011
Midges [Diptera: Chironomidae]					
Chironomus riparius	TGAI	25	0.622	1.328	MRID 44828402[1]
Chironomus riparius	NOS	25	1.6	3.2	Cleveland et al. 2002a
Chironomus riparius	NOS	25	1.6	N.S.	European Commission 2006
Shrimp [Mysida: Mysidae]					
Mysidopsis bahia	TGAI	28	84.2	173	MRID 44420602[1,2]

[1] See Appendix 6, Table A6-2 for reference to source of MRID summary.
[2] Specified as flow-through study.
[3] Specified as static renewal study.
[4] Up to 60 days at concentrations up to 6 µg a.i./L. See Section 4.1.3.3.2.1 for discussion.
[5] No organisms survived the 10 day exposure.
N.S.: Not specified. N.D. Not determined.

See Appendix 6, Table A6-2 for details.
See Section 4.1.3.3.2.1 for discussion.

Table 27: Terrestrial Nontarget Animals Used in Ecological Risk Assessment

MAMMALS [1]

Animal	Representative Species	BW[4]	Food Consumption[5]	Water Consumption
Small mammal	Mice	20	$2.514\ W^{0.507}$ [Eq 3-48]	$0.099\ W^{0.9}$ [Eq 3-17]
Larger mammal	Squirrels	400	$2.514\ W^{0.507}$ [Eq 3-48]	$0.099\ W^{0.9}$ [Eq 3-17]
Canid	Fox	5,000	$0.6167\ W^{0.862}$ [Eq 3-47]	$0.099\ W^{0.9}$ [Eq 3-17]
Large Herbivorous Mammal	Deer	70,000	$1.518\ W^{0.73}$ [Eq 3-46]	$0.099\ W^{0.9}$ [Eq 3-17]
Large Carnivorous Mammal	Bear	70,000	$0.6167\ W^{0.862}$ [Eq 3-47]	$0.099\ W^{0.9}$ [Eq 3-17]

BIRDS [2]

Animal	Representative Species	BW[4]	Food Consumption[5]	Water Consumption
Small bird	Passerines	10	$2.123\ W^{0.749}$ [Eq 3-36]	$0.059\ W^{0.67}$ [Eq 3-15]
Predatory bird	Owls	640	$1.146\ W^{0.749}$ [Eq 3-37]	$0.059\ W^{0.67}$ [Eq 3-15]
Piscivorous bird	Herons	2,400	$1.916\ W^{0.704}$ [Eq 3-38]	$0.059\ W^{0.67}$ [Eq 3-15]
Large herbivorous bird	Geese	4,000	$1.146\ W^{0.749}$ [Eq 3-37]	$0.059\ W^{0.67}$ [Eq 3-15]

INVERTEBRATES [3]

Animal	Representative Species	BW[4]	Food Consumption[5]
Honey bee [7]	*Apis mellifera*	0.000116	≈2 (1.2 to 4)[6]
Herbivorous Insects	Various	Not used	1.3 (0.6 to 2.2)

[1] Sources: Reid 2006; U.S. EPA/ORD 1993.
[2] Sources: Sibley 2000; Dunning 1993; U.S. EPA/ORD 1993.
[3] Sources: Humphrey and Dykes 2008; Reichle et al. 1973; Winston 1987
[4] Body weight in grams.
[5] For vertebrates, based on allometric relationships estimating field metabolic rates in kcal/day for rodents (omnivores), herbivores, and non-herbivores. For mammals and birds, the estimates are based on Nagy (1987) as adapted by U.S. EPA/ORD (1993). The equation numbers refer to U.S. EPA/ORD (1993). See the following table for estimates of caloric content of food items. For herbivorous insects, consumption estimates are based on fractions of body weight (g food consumed/g bw) from the references in Note 3.
[6] For honeybees, food consumption based on activity and caloric requirements.
[7] A surface area of 1.42 cm2 is used for the direct spray scenario of the honey bee. This value is based on the algorithms suggested by Humphrey and Dykes (2008) for a bee with a body length of 1.44 cm.

See data on food commodities in following table.
See Sections 4.2.2 and 4.2.3.2 for discussion.

Table 28: Diets: Metabolizable Energy of Various Food Commodities

Food Item	Animal Group	Caloric Value [1] (kcal/g bw)	Water Content [2]	Comment/Source(s)
Fruit	Mammals	1.1	0.77	See Footnote 3
	Birds	1.1	0.77	See Footnote 4
Fish	Mammals	4.47	0.70	Water content from Ali et al. (2005).
	Birds	3.87	0.70	Water content from Ali et al. (2005).
Insects	Mammals	4.47	0.70	Water contents from Chapman 1998 (p. 491). Typical ranges of 60-80%.
	Birds	4.30	0.70	Water contents from Chapman 1998 (p. 491). Typical ranges of 60-80%.
Vegetation (NOS)	Mammals	2.26	0.85	See Footnote 5
	Birds	2.0	0.85	See Footnote 5

[1] Metabolizable energy. Unless otherwise specified, the values are taken from U.S. EPA/ORD (1993), Table 3-1, p. 3-5 as adopted from Nagy 1987.

[2] From U.S. EPA/ORD (1993), Table 4-2, p. 4-14 unless otherwise specified.

[3] Based on a gross caloric value of 2.2 kcal/g bw (U.S. EPA/ORD 1993, Table 4-2). An assimilation factor for mammals eating fruit not identified. Use estimate for birds (see below).

[4] Based on a gross caloric value of 2.2 kcal/g bw (U.S. EPA/ORD 1993, Table 4-2) and an assimilation factor for the consumption of fruit by birds of 51% [2.2 kcal/g bw x 0.51 ≈ 1.1 kcal/g bw]

[5] Based on a gross caloric value of 4.2 kcal/g bw for dicot leaves (U.S. EPA/ORD 1993, Table 4-2). For birds, the value is corrected by an assimilation factor for the consumption leaves by birds of 47% [4.2 kcal/g bw x 0.47 = 1.974 kcal/g bw]

See Sections 4.2.2.3 for discussion.

Table 29: Estimate of Concentration of Spinosad in Nectar

Ratios of Concentration of Pollen to Nectar (Dively and Kamel 2012, Tables 1 and 2)

Pesticide	Treatment Method	Pollen (ng/g), Mean	Nectar (ng/g), Mean	Ratio
Imidacloprid	bedding Drench	4.9	0.4	0.08
	transplant (low)	36.7	5.7	0.16
	transplant (high)	60.9	7.4	0.12
	transplant-drip	80.2	11.2	0.14
Dinotefuran	transplant-drip	57.5	9.2	0.16
	two foliar	88.3	7.5	0.08
Thiamethoxam	transplant-drip	60	9.5	0.16
	two foliar	95.2	8.2	0.09

Analysis

Statistic	Value	Units
Mean Ratio	0.12	Unitless
Lower 5% bound of Ratio	0.099	Unitless
Upper 95% bound of Ratio	0.15	Unitless
Pollen Rate for Spinosad	8.96	mg/kg per lb/acre
Estimated Nectar Rates for Spinosad		
Mean	1.08	mg/kg per lb/acre
Lower 5% bound	0.89	mg/kg per lb/acre
Upper 95% bound	1.34	mg/kg per lb/acre

See Section 4.2.3.3.2 for discussion.

Table 30: Concentrations of Spinosad in Clay

One Application	**Top 12 inches**[1]	**Top 36 Inches**[1]
Central	0.37	0.122
Lower	0.36	0.121
Upper	0.38	0.127
Two Applications (6 day interval)	**Top 12 inches**[2]	**Top 36 Inches**[2]
Central	0.73	0.244
Lower	0.72	0.24
Upper	0.82	0.272

[1] Concentrations in units of mg a.i./kg soil expected at a unit application rate of 1 lb a.i./acre. The estimates are taken from Appendix 8 (Tables A8-2 and A8-3) for a single application and Appendix 9 (Tables A9-2 and A9-3) for two applications with a six day application interval.

See Section 4.2.3.4 for discussion

Table 31: Summary of toxicity values used in ecological risk assessment

Group/Duration Organism		Endpoint	Toxicity Value (a.i.)	Reference
Terrestrial Animals				
Acute				
Mammals (excluding canids)		Lowest Rat LD_{50} (3738 mg/kg) ÷ 10	370 mg/kg bw	Section 4.3.2.1.
Canids		NOAEL, subchronic, organ pathology	4.9 mg/kg bw	
Birds		Dietary (656 ppm) NOAEL, quail	200 mg/kg bw	Section 4.3.2.2
Honey Bee (contact)		NOAEL[1]	0.014 mg/kg bw	Section 4.3.2.4.1
Honey Bee (oral)		LD_{50} ÷ 10	0.041 mg/kg bw	Section 4.3.2.4.2
Phytophagous insect (oral)		Estimated dietary NOAEL	0.08 mg/kg bw	Section 4.3.2.4.3
Longer-term				
Mammals (excluding canids)		NOAEL, rats	10 mg/kg bw	Section 4.3.2.1
Canids		NOAEL, organ pathology	2.49 mg/kg bw	
Bird		NOAEL, quail and mallards, reproduction.	38.5 mg/kg bw	Section 4.3.2.2.
Aquatic Animals				
Acute				
Fish	Sensitive	Estimated NOAEC, carp	1.1 mg/L	Section 4.3.3.1
	Tolerant	Estimated NOAEC, trout	6.9 mg/L	
Invertebrates	Sensitive	Used chronic value	0.0005 mg/L	Section 4.3.3.3.1.1
	Tolerant	Mosquito,LC50 ÷20	0.023 mg/L	
Longer-term				
Fish	Sensitive	NOAEC, trout	0.498 mg/L	Section 4.3.3.1
	Tolerant	NOAEC, sheepshead minnow	1.15 mg/L	
Invertebrates	Sensitive	*Ceriodaphnia* NOAEC	0.0005 mg/L	Section 4.3.3.3
	Tolerant	Midge NOAEC	0.0842 mg/L	
Aquatic Plants				
Algae	Sensitive	*N. pelliculosa*, NOAEC	0.05 mg/L	Section 4.3.3.4
	Tolerant	*S. capricornutum*, NOAEC	4.3 mg/L	Section 4.3.3.4
Macrophytes	Sensitive	No identified		Section 4.3.3.4
	Tolerant	*Lemna*, NOAEC	1.86 mg/L	Section 4.3.3.4

[1] This is the NOAEL from the study reporting the lowest topical of LD_{50} of 0.0029 µg a.i./bee (Hoxter et al. 1992). Other toxicity studies in honeybees report substantially higher topical LD_{50} values (i.e., by factors of up to 340). See Section 4.3.2.4.1 for discussion.

See Section 4.3.1 for initial discussion.

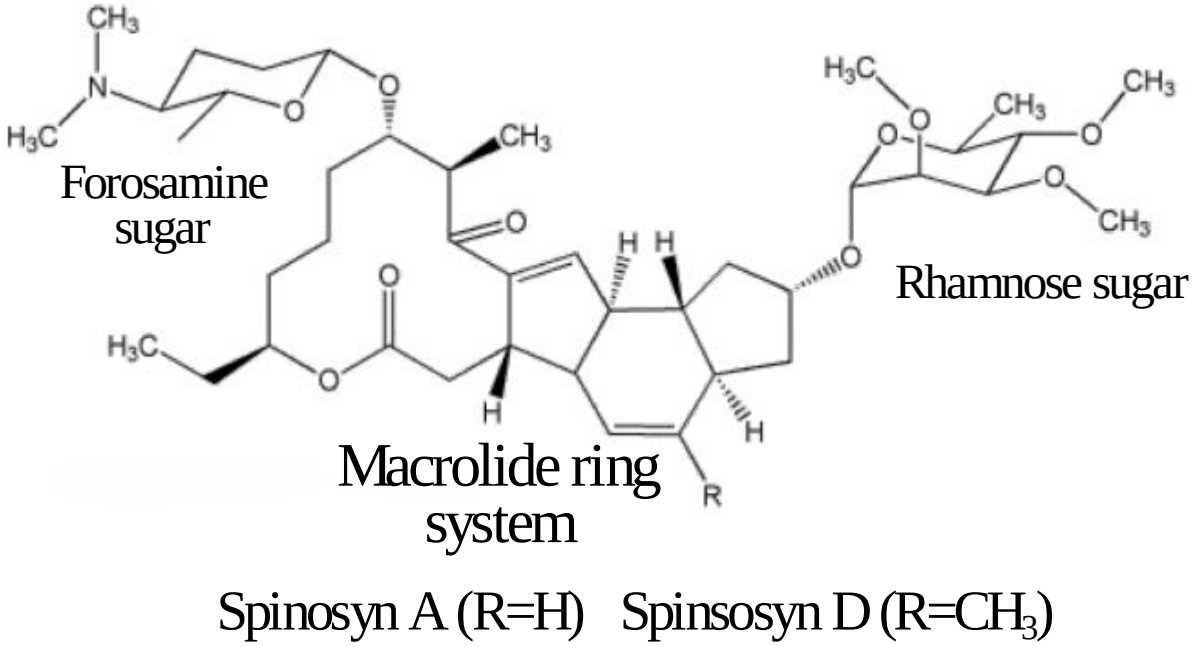
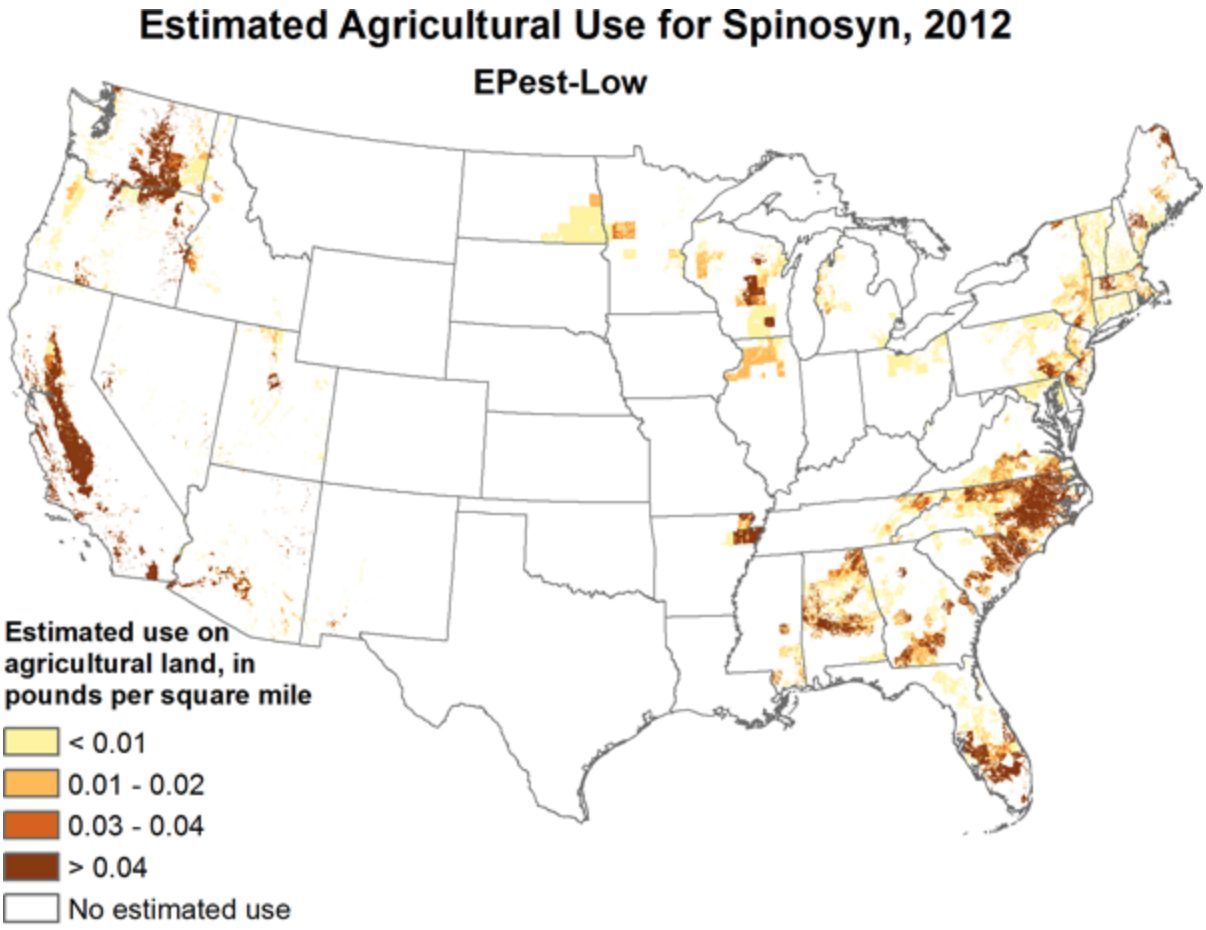


Figure 1: Structures of Spinosyn A and D

Source: Modified from EFSA 2011, p. 7



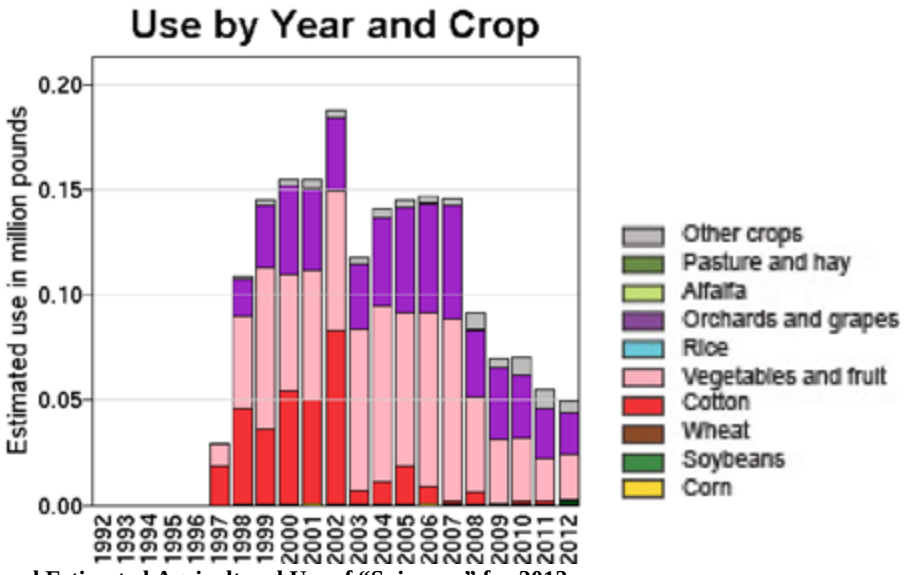
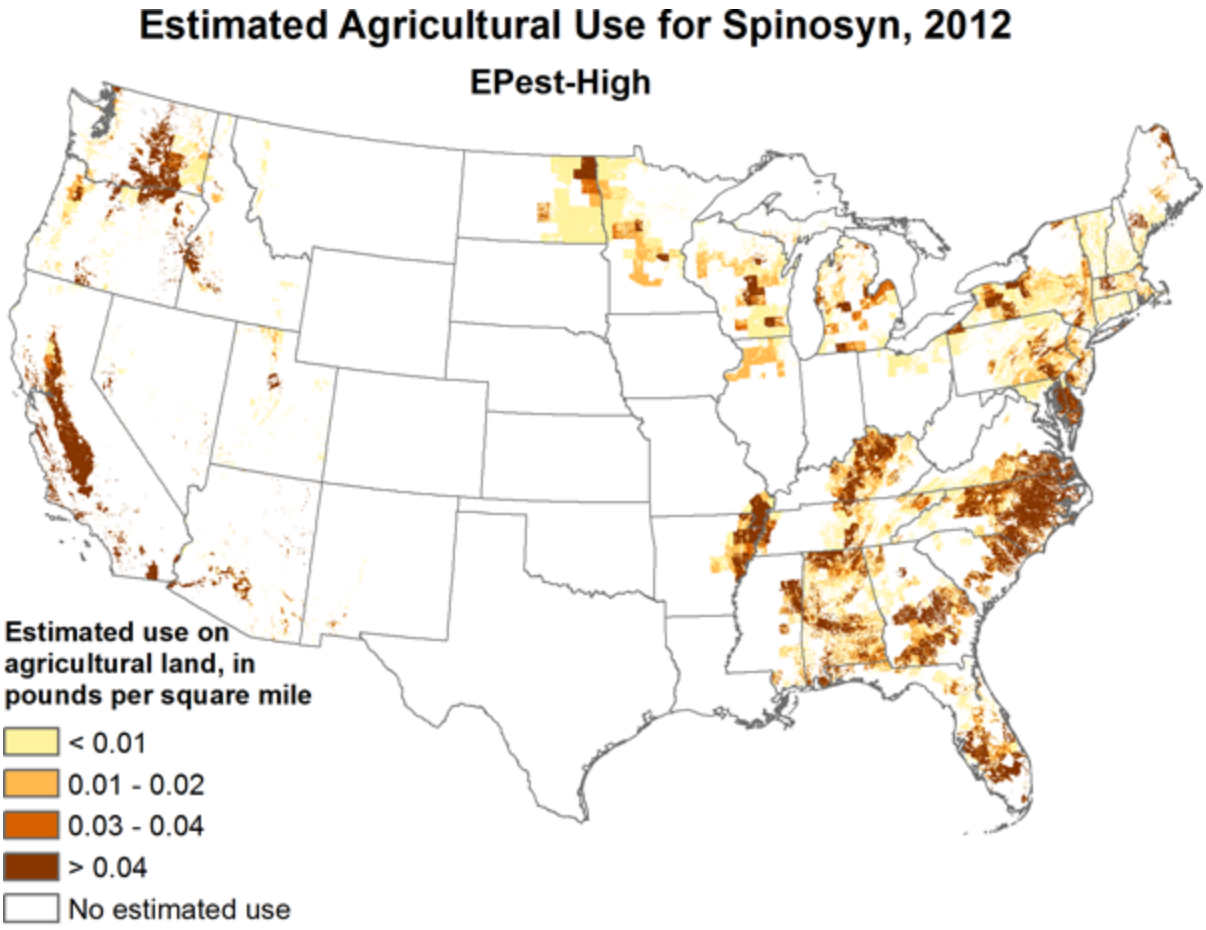


Figure 2: Lower Bound Estimated Agricultural Use of "Spinosyn" for 2012

Source: USGS(2015)
See Section 2.5 for discussion.



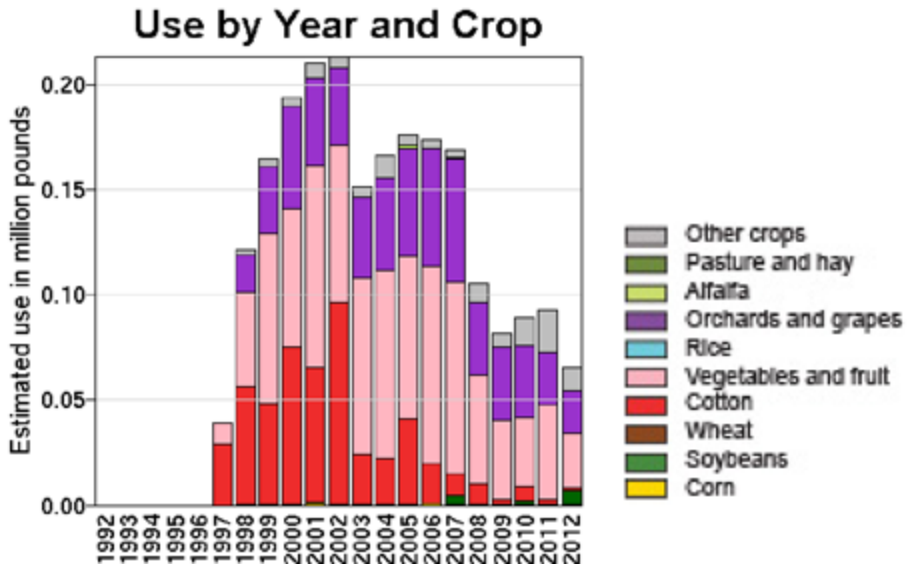


Figure 3: Upper Bound Estimated Agricultural Use of "Spinosyn" for 2012

Source: USGS(2015)
See Section 2.5 for discussion.

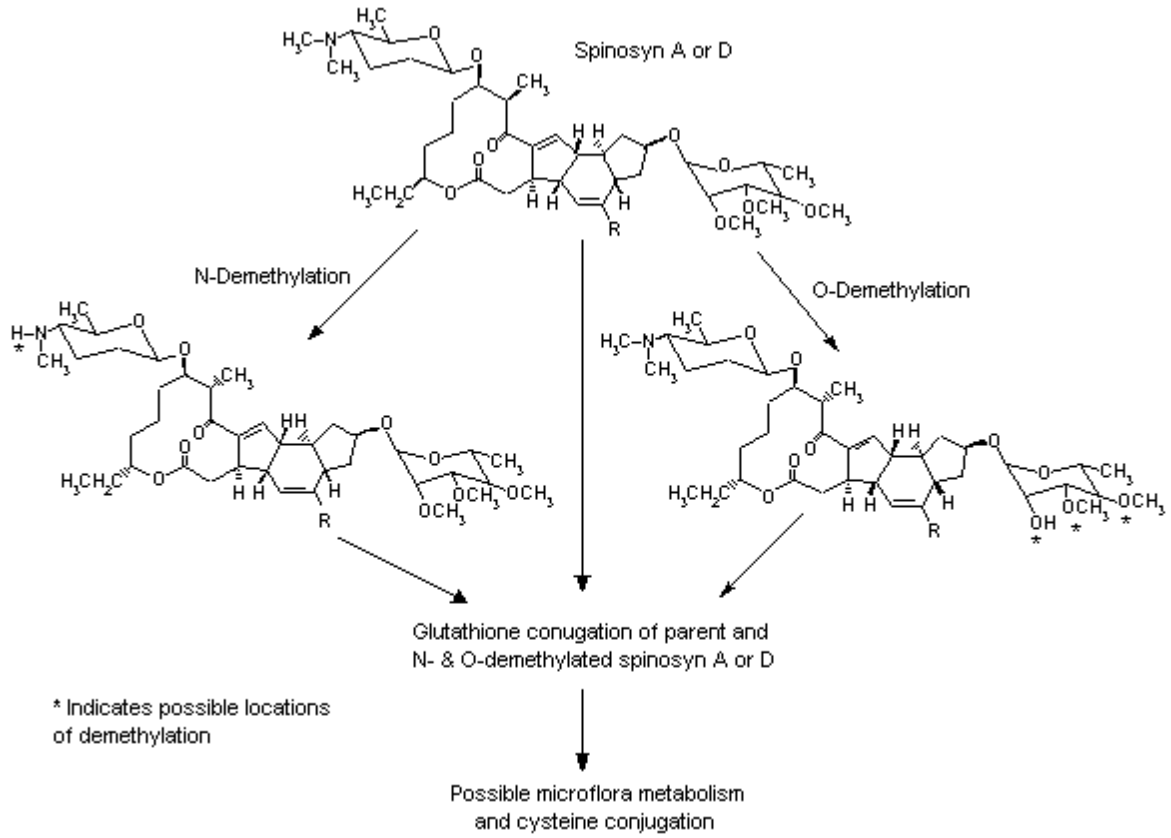


Figure 4: Metabolic pathway in mammals for spinosad

Source: FAO/WHO 2001.
See Section 3.1.3.1 for discussion.

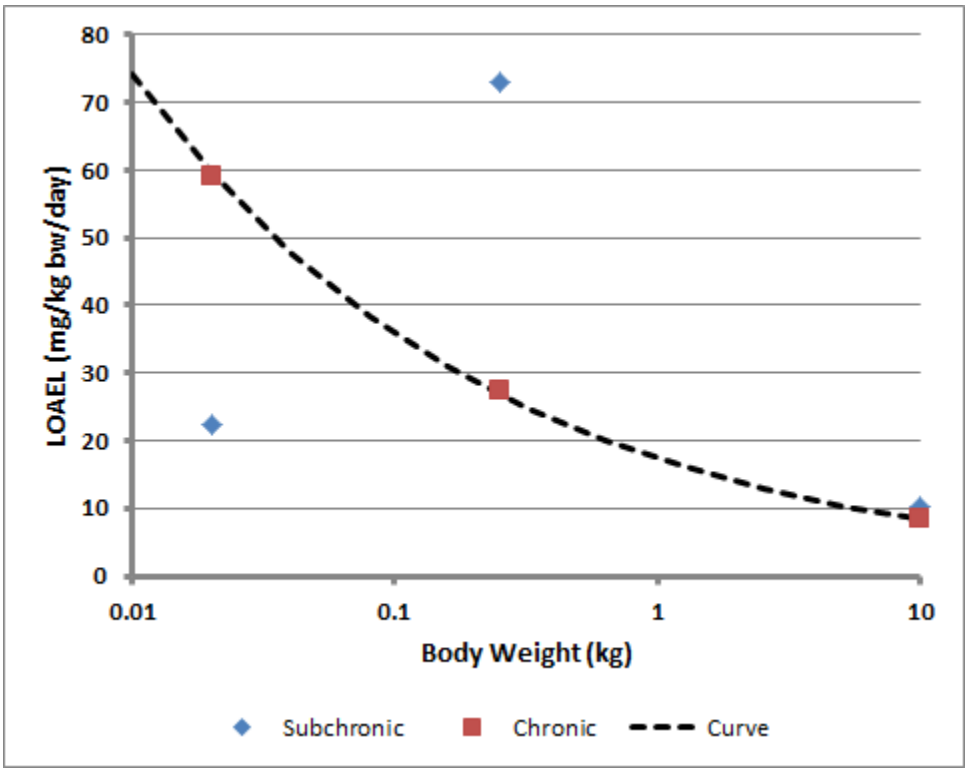


Figure 5: Mammalian Sensitivities to Spinosad

See Section 3.1.5 for initial discussion.
See Table 6 for data.

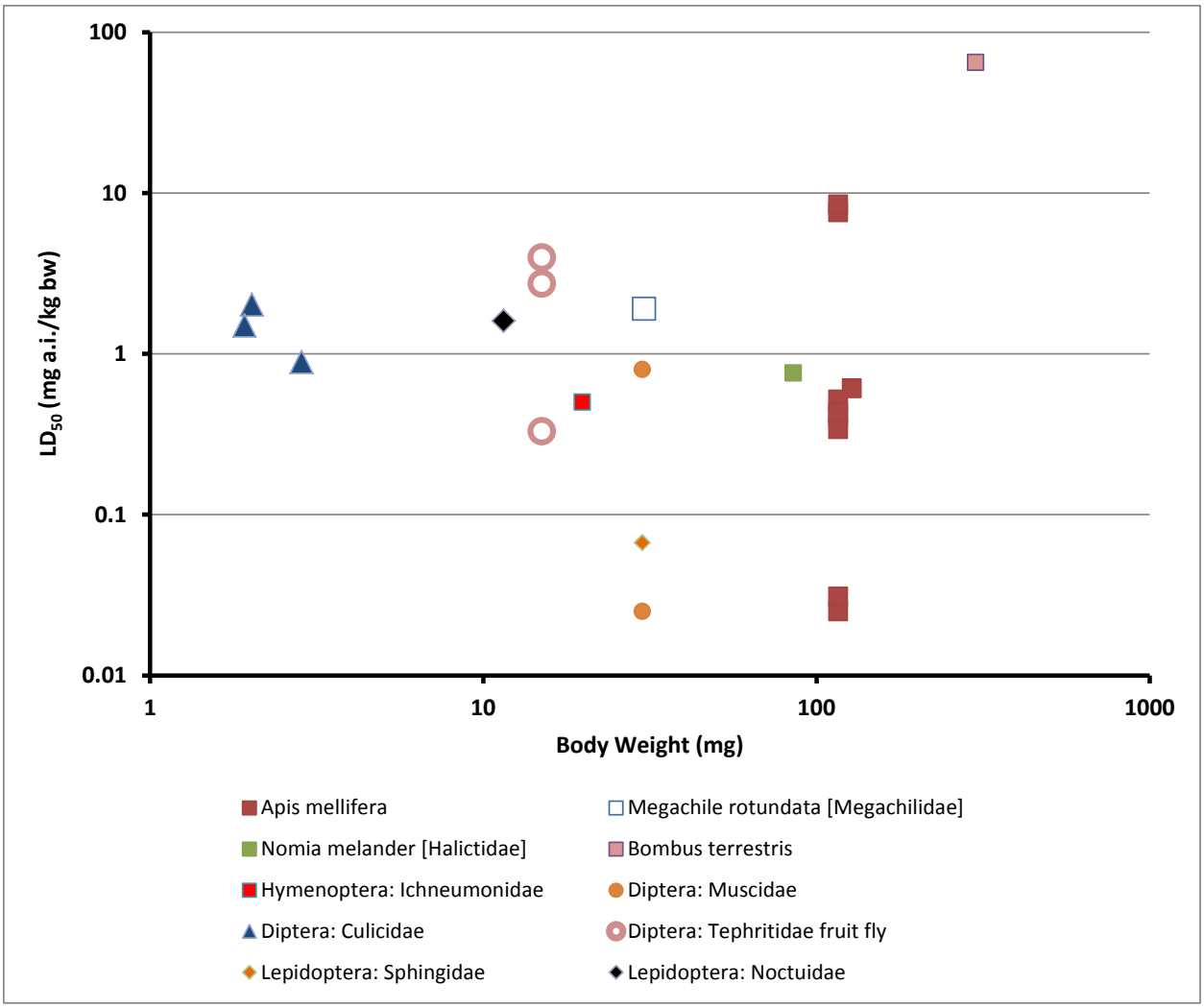


Figure 6: Topical LD_{50} Values in Insects by Body Weight

See Table 19 for data.
See Section 4.1.2.4.3.1 for discussion.

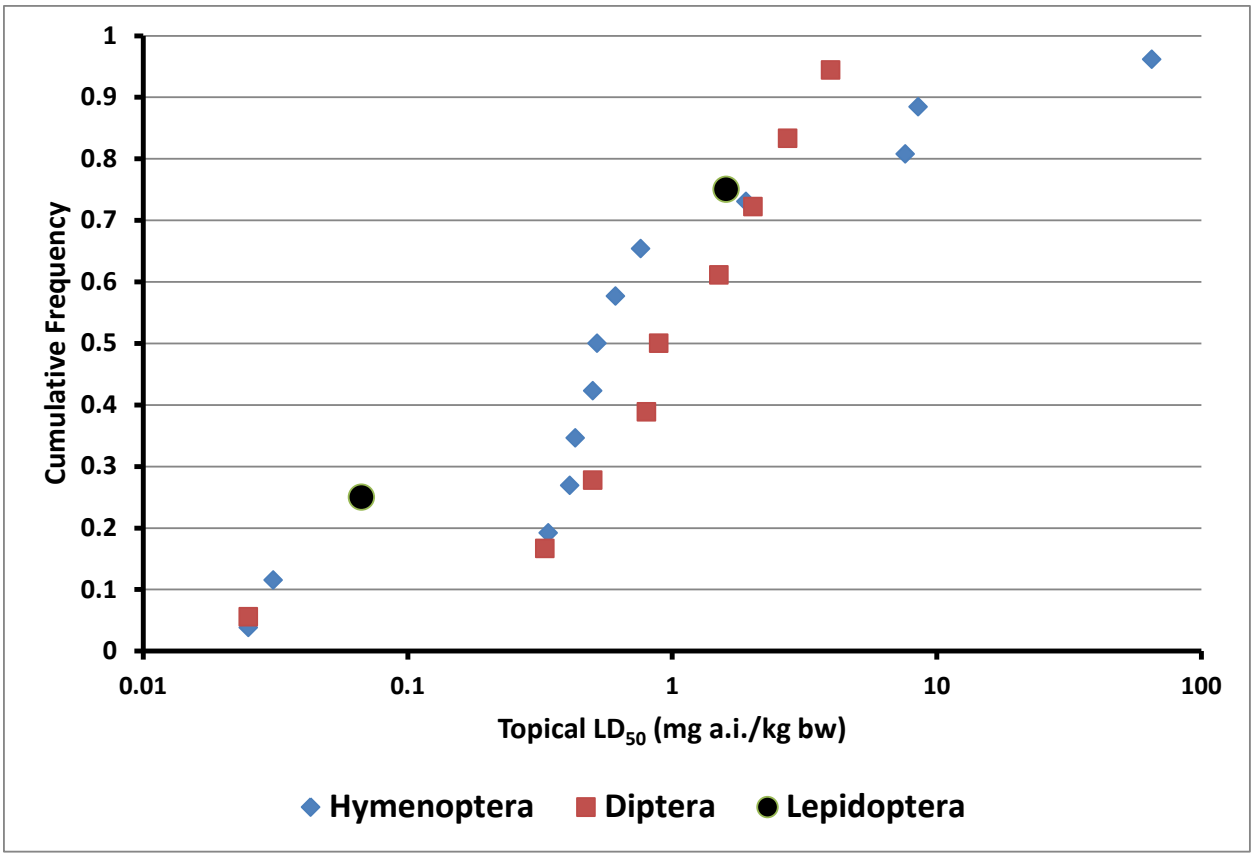
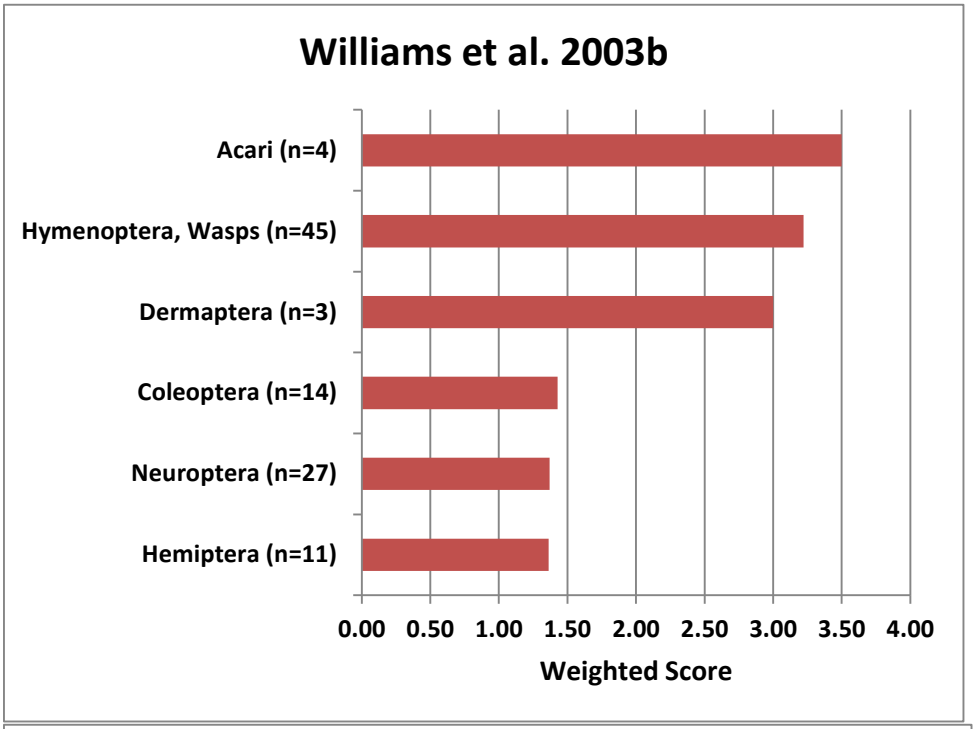


Figure 7: Sensitivity Distributions by Insect Order of Contact LD_{50} Values

See Table 19 for data.
See Section 4.1.2.4.3.2 for discussion.



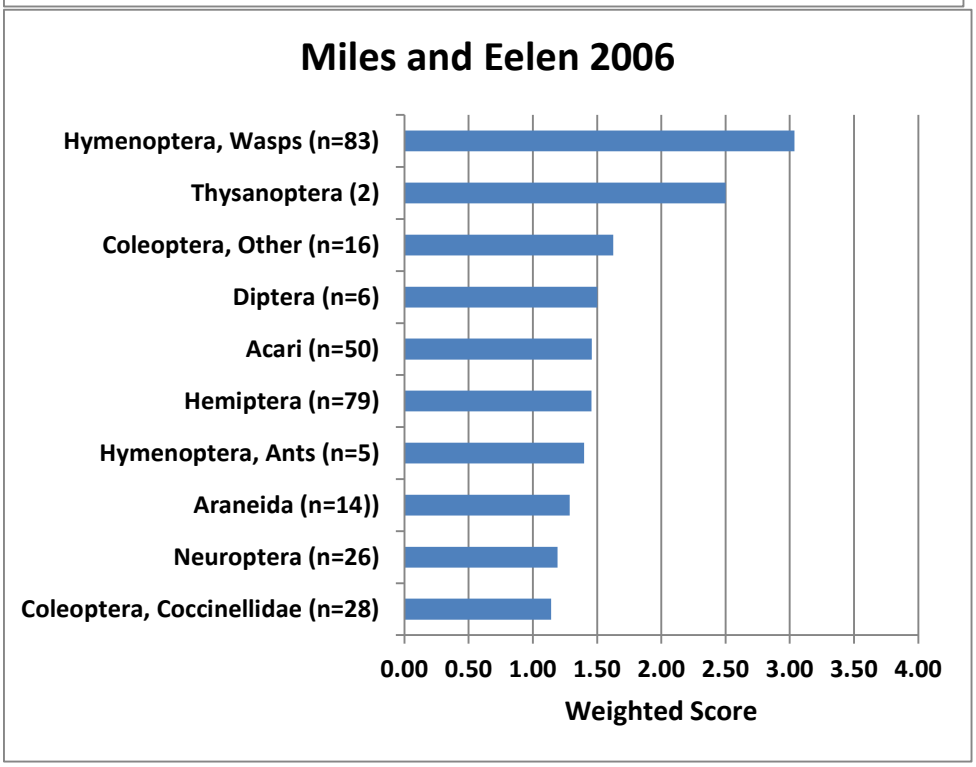


Figure 8: Weighted IOBC Scores for Spinosad

See Table 21 for data.
See Section 4.1.2.4.4.1 for discussion.

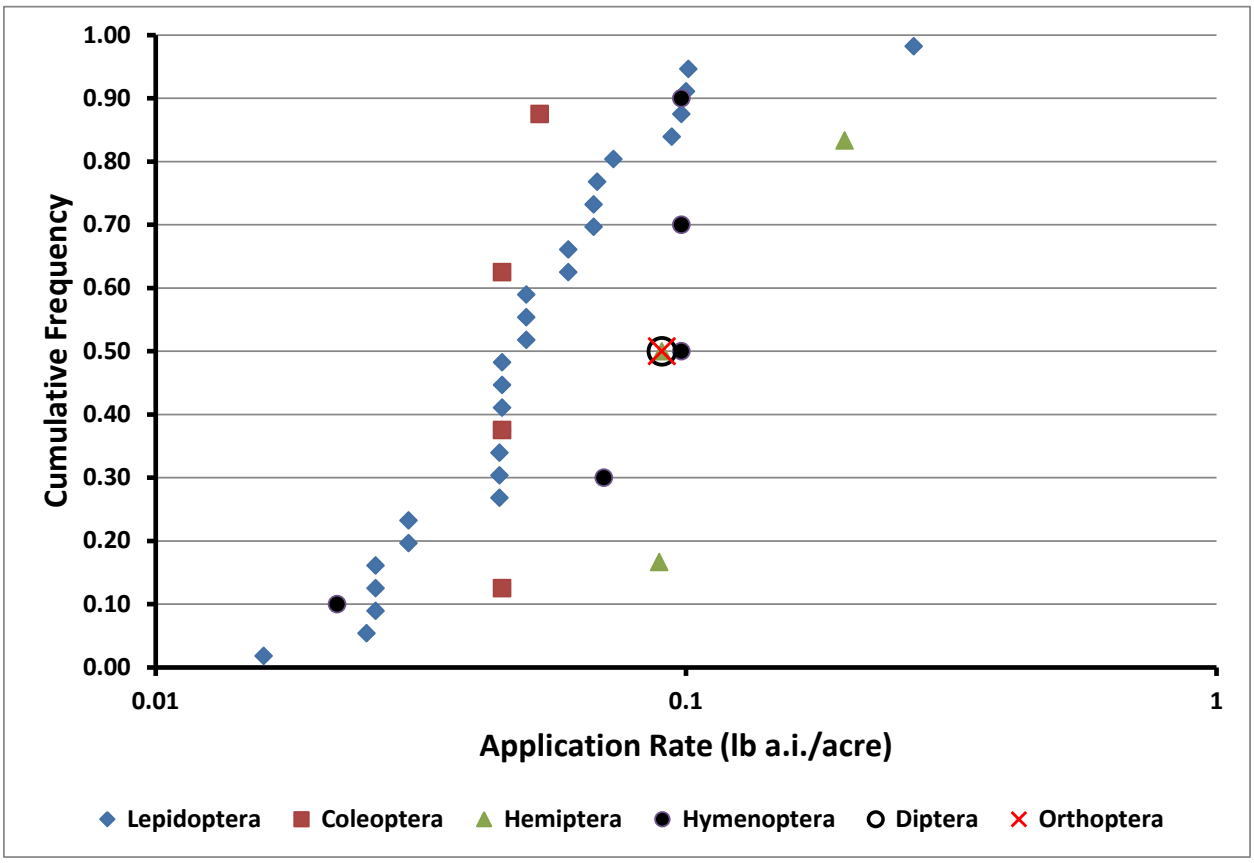


Figure 9: LOAECs from Field Studies for Orders of Terrestrial Insects

See Table 23 for data.
See Section 4.1.2.4.5 for discussion.

Appendix 1: Toxicity to mammals.

Note: Except as otherwise noted, full studies or DERs of MRID studies were not available for the current risk assessment. Information is taken with little or no modifications from the cited EPA risk assessments. DERs, when available, are cited by author and date followed by the MRID number. All MRID studies classified by EPA as *Acceptable* except as otherwise noted.
Designations such as 6.4:1::A:D refer to the ratio of spinosyn A to spinosyn D.
The 44% a.i. formulation cited to U.S. EPA/OPP/HED 1997b appears to be the Tracer formulation, 44.2% a.i.
Most of the registrant studies are also summarized in WHO (2008, 2011).

Table A1-1: Acute Oral LD_{50} Values

Species	Compound	Response	Reference
Gavage			
Rats, Fischer 344, 5 per sex per group. 8-9 weeks old.	Spinosad (78.2% a.i.) XDE-105) Doses: 0 and 2000 mg/kg bw	LD_{50}: >2000 mg/kg bw Category III Clinical Signs: soft stool, hypoactivity and hunched posture on Day 1-2 in malls. Posterior soiling and poor grooming in females on Days 1-4. Working Note: This study is classified as Supplementary in EPA's DER.	Wright et al. 1992 MRIDs 43770701 and 43414515 U.S. EPA/OPP/HED 1997b, 2009a
Rats	Spinosad (TGAI, 88-90.4%) Doses: 0 and 5000 mg/kg bw	LD_{50}: Males: 3738 mg/kg bw Females: >5000 mg/kg bw Working Note: The DER for Wright et al. 1992 notes that the LD_{50} reported in this study probably incorporates the data from Wright et al. 1992 and that spinosad should be classified as Category III in male rats.	MRID 43414515 U.S. EPA/OPP/HED 1997b The definitive LD_{50} in males is used in current risk assessment as the basis for the acute toxicity value to non-canid mammals.
Rats, Fischer 344, 5 per sex	Spinosad (XDE-105, 87.9% a.i.) 5,000 mg a.i./kg bw 2 week observation period.	Mortality in 4 males (2 each on Days 7 and 8). Mortality in 1 female on Day 8 LD_{50}: Males: 3738 mg/kg bw Females: >5000 mg/kg bw	Gilbert et al. 1994 MRID 43414515 Working Note: Minor discrepancies in experimental detail from above EPA summary but an identical LD_{50}s for males and females.

Appendix 1: Toxicity to mammals *(continued)*

Species	Compound	Response	Reference
Gavage			
Rats, Fischer 344	Spinosad (96.3 % a.i.; 46.1% spinosyn A and 50.2 % spinosyn D)	LD_{50}: Males: 3738 mg/kg bw Females: >5000 mg/kg bw	FAO/WHO 2001, citing Stebbins and Brooks 1999a
Rats, Fischer (acute neurotoxicity), 10 per sex per dose	Spinosad (EXE-105, 87.9% a.i.) Single Doses: 0, 200, 630, or 2000 mg/kg Observation Period: 15 days. FOB (functional observational battery) assays conducted on Days -1 (pre-dosing), 1 (5-6 hours post-dosing), 8, and 15.	NOAEL: 2000 mg/kg in males and females. LOAEL: Not determined. Transient body weight decrease on Day 2 after dosing. Not apparent by Days 8-15 (p. 7 of DER) Working Note: The DER is detailed and supports the EPA assessment of no adverse effects at an doses.	Albee et al. 1994 MRIDs 43557501 U.S. EPA/OPP/HED 2009a
Mice, CD-1, 5 per sex	Spinosad (XDE-105, 87.9% a.i.) 6,000 mg a.i./kg bw 2 week observation period.	Mortality in 1 male and 2 female mice on Days 11-12. LD_{50}: >5000 mg/kg bw Working Note: The >5000 mg/kg bw is a conventional reporting term from EPA. The LD50 appears to be >6000 mg/kg bw .	Gilbert et al. 1994 MRID 43414515
Mice, CD-1	Spinosad, 87.9 % a.i.	LD_{50}: Males: 6100 mg/kg bw Females: 7100 mg/kg bw	Gilbert and Yano 1996 as summarized in FAO/WHO 2001

See Section 3.1.4 for general discussion as well as limited toxicity data in cats and dogs from veterinary efficacy studies.

Table A1-2: Subchronic and Chronic Oral Toxicity Studies

Organism	Agent/Exposure	Response	MRID, Study Date, Classification
Subchronic			
Dogs, beagle	Spinosad, TGAI, 88% a.i. Dietary Concentrations: 0, 150, 300, or 1350/900 (males), 900 (females) ppm. Doses (M/F): Male: 0, 4.89, 9.73, or 33.4/22.5 mg/kg bw Female:0, 5.38, 10.47, or 29.9 mg/kg bw Duration: 13 weeks **Working Note: The high dose in male dogs was reduced on day 38 of study.**	NOAEL: 4.89 mg/kg/day in males; 5.38 mg/kg/day in females. Average of NOAELs: 5.135 mg/kg bw/day LOAEL: 9.73 mg/kg/day in males; 10.47 mg/kg/day in females (average of 10.1 mg/kg bw/day) based on microscopic changes in a variety of tissues, clinical signs of toxicity, decreases in mean body weights and food consumption, and biochemical evidence of anemia and possible liver damage	MRID 43444102 U.S. EPA/OPP/HED 1997b, 2009a Working Note: The NOAEL of 4.89 mg/kg bw/day in male dogs is rounded to 4.9 mg/kg bw and used as the basic for the short-term incidental oral dose-response assessment with a MOE of 100.
Mice, CD-1 strain	Spinosad, TGAI, 88% a.i. Dietary Concentrations: 0, 0.005, 0.015, 0.045, or 0.12% 0, 50, 150, 450, or 1200 ppm Doses: 0, 7.5, 22.5, 67.5, or 180 mg/kg/day Duration: 90 days.	NOAEL: 7.5 mg/kg/day in males and females. LOAEL: 22.5 mg/kg/day in males and females; based on cytoplasmic vacuolation of lymphoid organs, liver, kidney, stomach, female reproductive tract, and epididymis. Other tissues less severely affected are heart, lung, pancreas, adrenal cortex, bone marrow, tongue, and pituitary gland. High Dose (180 mg/kg bw/day): Terminated group after 6 weeks due to mortality (3/10 Males and 2/10 females).	MRID 43566602 U.S. EPA/OPP/HED 1997b, 2009a

Organism	Agent/Exposure	Response	MRID, Study Date, Classification
Mice, CD-1 strain, 10 per sex per dose	Spinosad, TGAI, 77.6% a.i. Dietary Concentrations: 0, 0.005, 0.015, 0.045, or 0.12% 0, 50, 150, 450, or 1200 ppm Doses (based on bw and estimated consumption of 4.2 g/day [2.1 g/day in high dose]): Males: 0, 6.0, 17.9, 57.2, or 109.7 mg/kg/day Females: 0, 8.1, 23.1, 71.5, or 141.9 mg/kg/day Duration: 13 weeks (91 days) except for high dose, which was terminated on Day 44.	NOAEL: 50 ppm, 6/8.1 mg/kg/day (M/F). LOAEL: 150 ppm, 17.9/23.1 mg/kg bw/day (M/F); responses consistent with MRID 43566602.	Stebbins et al. 2002, Study 1 Working Note: Except for minor differences in reporting and estimates of daily doses, this study appears to be identical to MRID 43566602.
Rats, adult, male, Sprague-Dawley, 160-185 g, 10 per dose	Spinosad (Tracer® formulation, 24% a.i., SC) In wheat grain Dietary Concentrations: 0, 8, and 16 ppm grain. Duration: 90 days Food consumption not reported.	No overt signs of toxicity or mortality. Dose-related increase in number of aberrations in rat bone marrow at 8 and 16 ppm (Table 2). Increase in DNA content of rat livers at 16 ppm but not 8 ppm. Decrease in total protein in rat livers at both 8 and 16 ppm. Dose related decrease in RBCs and hematocrit and increase in white blood cell counts at high dose (Table 1 of study).	El-Hoda et al. 2012 Egypt
Rats, Fischer 344, 10 per sex per dose.	Spinosad, 77.6% a.i., 5:1::A:D. Dietary Concentrations: 0, 0.05, 0.1, 0.2, or 0.4% Dietary Concentrations: 0, 500, 1000, 2000, 4000 ppm. Doses: Male: 0, 33.9, 68.5, 133.5, or 273.1 mg/kg/day Female: 0, 38.8, 78.1, 151.6, or 308.2 mg/kg/day Duration: 90 days Working Note: FAO review rounds doses to two significant places.	NOAEL: 33.9 mg/kg/day in males; 38.8 mg/kg/day in females. Average of NOAEL: 36.35 mg/kg bw/day. LOAEL: 68.5 mg/kg/day in males; 78.1 mg/kg/day in females based on adrenal cortical vacuolation in males, lymph node histiocytosis in both sexes. Average LOAEL of about 73.3 mg/kg bw/day. Increase in thyroid weights but not statistically significant (FAO summary). High Dose (4000 ppm): Discontinued on Day 44 due to deaths in 5/10 male and female rats.	MRID 43566601 U.S. EPA/OPP/HED 2009a Also summarized in FAO/WHO 2001.

Organism	Agent/Exposure	Response	MRID, Study Date, Classification
Rats, 10 per sex per dose. (neurotoxicity)	Spinosad (TGAI, XDE-105, 87.9%) Dietary Concentrations: 0, 0.003, 0.006, 0.012 or 0.06% Dietary Concentrations: 0, 30, 60, 120, 600 ppm Doses: Males: o, 2.2, 4.3, 8.6, and 42.7 mg/kg bw/day Females: 0, 2.6, 5.2, 10.4 and 52.1 Duration: 13 weeks. Working Note: DER gives high dose in females as 52.5 mg/kg bw/day in methods section but 52.1 mg/kg bw/day as NOAEL for females.	No effects in the functional observational battery (FOB), motor activity, or histological observations of the nervous system. NOAEL: 42.7 mg/kg/day in males; 52.1 mg/kg/day in females. LOAEL: Not determined.	Wilmer et al. 1993 MRID 43557504 U.S. EPA/OPP/HED 1997b, 2009a Working Note: This study is also summarized in U.S. EPA/OPP/HED 2009a as MRID 43557502.
Chronic			
Dogs, beagle, 4 per sex per dose	Spinosad (TGAI, XDE-105, 87.2%) Dietary Concentrations (M/F): 0, 50/60, 100/120, or 300/360 ppm Doses (M/F): Males: 0, 1.44, 2.68, or 8.46 mg/kg/day Females: 0, 1.33, 2.72, or 8.22 mg/kg/day Duration: 52 weeks	NOAEL: 2.68 mg/kg/day in males, 2.72 mg/kg/day in females. Average NOAEL: 2.7 mg/kg bw/day. LOAEL: 8.46 mg/kg/day in males; 8.22 mg/kg/day in females (average LOAEL of 8.34 mg/kg bw/day) based on increases in serum alanine aminotransferase, aspartate aminotransferase, and triglycerides levels, and the presence of tissue abnormalities, including vacuolated cell aggregations, arteritis, and glandular cell vacuolation (parathyroid). DER notes a 160% increase in thyroid weights in female dogs in high dose but no pathology.	Harada 1995 MRID 43701504 U.S. EPA/OPP/HED 1997b, 2009a Working Note: The NOAEL of 2.68 mg/kg bw/day in male dogs used as the basic for the chronic RfD in U.S. EPA/OPP/1997b. The more recent RfD based on Spinetoram (see below) is used in current Forest Service risk assessment.
Dogs	**Spinetoram** Dietary Concentrations: 0, 50, 100, or 200 ppm Doses Male: 0, 1.57, 2.96, and 5.36 mg/kg/day Female: 0, 1.31, 2.49, and 5.83 mg/kg bw/day. Duration: 1 year	NOAEL = 100 ppm (2.49 mg/kg/day in females/2.96 mg/kg/day in males). LOAEL = 200 ppm (5.36 mg/kg/day in males/5.83 mg/kg/day in females) based on arteritis and necrosis of the arterial walls of the epididymides in males, and the thymus, thyroid, larynx and urinary bladder in females	MRID 47011901 U.S. EPA/OPP/HED 2009a Working Note: The NOAEL of 2.49 mg/kg bw/day in female dogs used as the basic for the chronic RfD in U.S. EPA/OPP/2009a.

Appendix 1: Toxicity to mammals *(continued)*

Organism	Agent/Exposure	Response	MRID, Study Date, Classification
Mice, CD-1, 50 per sex per dose.	Spinosad, TGAI, XDE-105, 88% a.i., 6.4:1::A:D. Dietary Concentrations: 0, 25, 80, or 360 ppm Doses: (M/F): Male: 0, 3.4, 11.4, or 50.9 mg/kg/day. Female: 0, 4.2, 13.8, or 67.0 mg/kg/day. Duration: Up to 18 months. Interim sacrifices at 3 and 12 months.	NOAEL: 11.4 mg/kg/day in males, 13.8 mg/kg/day in females. Average of NOAELs: 12.6 mg/kg bw/day. LOAEL = 50.9 mg/kg/day in males; 67.0 mg/kg/day in females (average = 59 mg/kg bw/day) based on decreased weight gains, increased mortality, the hematologic effects, and the gross finding of increased thickening of the gastric mucosa in females and the histologic changes in the stomach of males. No evidence of carcinogenicity. Working Note: DER states that vacuolation in the organs was only slight and the severity of the lesions did not increase with time. This is well-documented in Tables 5-7.	Bond et al. 1995a MRID 43701505 U.S. EPA/OPP/HED 1997b, 2009a Working Note: Classified as Supplemental in DER and HED 1997b but upgraded to Acceptable/Guideline in HED 2009a. Also published in Stebbins et al. 2002.
Mice, CD-1, 60 per sex per dose	Spinosad Dietary Concentrations: 0, 0.0008, or 0.024% Dietary Concentrations: 0, 8, 240 ppm Doses: (M/F): Male: 0, 1.1, or 32.7 mg/kg/day. Female: 0, 1.3, or 41.5 mg/kg/day. Duration: 18 months	NOAEL not established. LOAEL = 1.1 mg/kg/day in males; 1.3 mg/kg/day in females. No evidence of carcinogenicity. **Working Note: This study is not discussed or explicitly discounted in EPA risk assessments although the study is classified as *Acceptable/Guideline.* FAO/WHO (2001) indicates that only *limited investigations were carried out* on the low dose group. No pathology is reported.**	MRID 44123601 U.S. EPA/OPP/HED 2009a; 2010b Also summarized in FAO/WHO (2001) as Bond et al. 1996.

Organism	Agent/Exposure	Response	MRID, Study Date, Classification
Rats, Fischer 334, 50 per sex per dose. Plus interim sacrifice group of 15 per sex per dose. See entry below.	Spinosad, XDE-105, 88% a.i., 6.4:1::A:D. Dietary Concentrations: 0, 0.005, 0.02, 0.05, or 0.1% Dietary Concentrations: 0, 50, 200, 500, or 1000 ppm Doses: Males: 0, 2.4, 9.5, 24.1, or 49.4 mg/kg/day. Females: 0, 3.0, 12.0, 30.3, or 62.8 mg/kg/day. Duration: 24 months	<u>U.S. EPA/OPP/HED 2009a</u> NOAEL = 9.5 mg/kg/day in males, 12.0 mg/kg/day in females. Average of NOAELs: 10.75 mg/kg bw/day. LOAEL = 24.1 mg/kg/day in males; 30.3 mg/kg/day in females based on vacuolation of the epithelial follicular cells of the thyroid in both sexes. No evidence of carcinogenicity. Average LOAEL of about 27.3 mg/kg bw/day. <u>EPA Data Evaluation Record</u> NOAEL: 3 mg/kg bw/day LOAEL: 9.5 mg/kg bw/day based on vacuolation of epithelial follicular cells of the thyroid in both sexes. High Dose: Significant increase in mortality and decrease in body weight in both males and females (Tables 3 and 4 of DER). Working Note: Executive summary of DER classifies 50 ppm as a NOAEL and 200 ppm as LOAEL based on thyroid pathology (epithelial follicular vacuolation). This is supported by narrative in DER (Tables 9A and 9B, pp. 19-20). In addition, FAO/WHO (2001) also classifies 50 ppm as a NOAEL and 200 ppm as a LOAEL. This discrepancy does not impact the RfD (based on NOAEL of 2.49 mg/kg bw/day in dogs). Chronic phase of study classified as Supplementary in DER.	Bond et al. 1995b MRIDs 43701507 and 43710503 U.S. EPA/OPP/HED 2009a Working Note: This study is not detailed in HED 1997b. The DER is dated March, 1996. Also published in Yano et al. 2002 and is summarized in detail in FAO/WHO 2001.

Appendix 1: Toxicity to mammals *(continued)*

Organism	Agent/Exposure	Response	MRID, Study Date, Classification
Rats (neurotoxicity), 10 per group for FOB and 5 per group for neuropathology.	Spinosad (TGAI, 87.2%) Dietary Concentrations: 0, or 0.1% Dietary Concentrations: 0 or 1000 ppm Doses (M/F): 0/0 or 46.0/57.0 mg/kg bw/day Duration: 1 year FOB Observations at 3, 6, 9, and 12 months. Neuropathology at 12 months.	NOAEL (neurotoxicity): 46.0 mg/kg/day in males; 57.0 mg/kg/day in females. LOAEL (neurotoxicity): Not determined. Thyroid pathology noted: moderate inflammation and significant increases in absolute and relative thyroid weights.	Spencer and Yano 1995 MRID 43701507 and 43701503 U.S. EPA/OPP/HED 2009a Working Note: Part of chronic toxicity study detailed above as MRIDs 43701507 and 43710503

See Section 3.1.5 for discussion.

Table A1-3: Reproductive and Developmental Studies

Species	Exposure	Response	MRID(s), (Year), Classification
Developmental			
Rabbit, mated females, New Zealand White, 20 per dose	Spinosad, TGAI, XDE-105, 88.06%, ≈6.3:1::A:D Gavage Doses: 0, 2.5, 10, or 50 mg/kg/day on gestation days (GD)7-19.	High Dose: Maternal effects (decreased defecation (in 6/20 animals compared with 2/10 in the control group), decreased body weight gain (28% less than that for the control group during gestation days 7 to 10), and reduced food consumption (the high dose group consumed an average amount that was 74% of the control group value). Difference in body weights (not body weight gain) with respect to control groups was only 1-2% -- i.e., 3.3253 kg in controls and 3.2953 kg in high dose on GD 28. NOAEL: 50 mg/kg bw/day (maternal and developmental). Working Note: One death in high dose group on GD 9 attributed to gavage error. The decreased defecation is not significant using Fisher's Exact test (p=0.117558). In DER, the EPA reviewer notes that the effect on body weight does not appear to be *toxicologically significant*.	Vedula et al. 1994 MRIDs 43414521 and 43770703 (range-finding) U.S. EPA/OPP/ HED 1997b, 2009a Also published in Breslin et al. 2000.
Rats, female, mated, Sprague-Dawley, 30 per dose.	Spinosad TGAI, XDE-105, 88.06%, 6.4:1::A:D Gavage Doses: 0, 10, 50, or 200 mg/kg/day Duration: Gestation Days 6 to 16.	Maternal Effects (marginal): Decreased body weight gain and body weight for 1 day at the highest dose. NOAEL: 200 mg/kg bw/day (maternal and developmental). Given the higher sensitivity of rabbits (see above), the DER classifies this study as *Core*, even though no frank maternal toxicity was noted.	Liberacki et al. 1993 MRIDs 43557505 and 43770702 (range-finding) U.S. EPA/OPP/ HED 1997b, 2009a Also published in Breslin et al. 2000. Abstract published in Marty et al. 1998

Species	Exposure	Response	MRID(s), (Year), Classification
Reproduction			
Rat, Sprague Dawley, 6 weeks old (P_1) 30 per sex per dose	Spinosad, 88% a.i., 6.4:1::A:D Dietary Concentrations: 0, 0.005, 0.02, or 0.2% Initial Dietary Concentrations: 0, 50, 200, 2000. Adjusted over time to maintain constant mg/kg bw/day doses. Doses: 0, 3, 10, or 100 mg/kg/day Durations: P_1: 10 weeks F_{1a} (P_2): 12 weeks. F_{1b} and F_2: not mated or otherwise exposed following birth.	Parental/Systemic NOAEL: 10 mg/kg/day. LOAEL: 100 mg/kg/day based on increases in heart, kidney, liver, spleen, and thyroid weights (increased in both sexes), corroborative histopathology in the spleen and thyroid (both sexes), heart and kidney (males only), and histopathologic lesions in the lungs and mesenteric lymph nodes (both sexes), stomach (females only), and prostate. Body weight reduction (2-9%) in both P_1 and P_2 adults (see Figure 2 in Hanley et al. 2002). Thyroid hormone (thyroxin, T_4) levels: No effects in P_2 males or females at any doses (Table 4 of Hanley et al. 2002). U.S. EPA/OPP/HED (2009a, p. 5) indicates … *increased levels of thyroid-stimulating hormone (TSH) and decreased levels of T_4.* Reproduction NOAEL: 10 mg/kg/day. LOAEL: 100 mg/kg/day based on increased incidence of dystocia and/or vaginal bleeding after parturition with associated increases in mortality in the dams. These effects resulted in the death of 5/30 P_2 females. Offspring NOAEL: 10 mg/kg/day. LOAEL: 100 mg/kg/day based on decreases in litter size (F_2 only), survival and body weights. Working Note: The publication by Hanley et al. (2002, p. 150 and Table 5) provides a much more detailed discussion of effects on offspring. Adverse effects on the offspring appear to be clearly secondary to maternal toxicity.	Breslin et al. 1994 MRIDs 43701506 U.S. EPA/OPP/HED 1997b, 2009a Also published in Hanley et al. 2002

Table A1-4: Skin Irritation and Sensitization Studies

Species	Exposure	Response	Reference
Skin Irritation[1]			
Rabbit	Spinosad (TGAI, 88-90.4%)	Not a skin irritant. Category IV	MRID 43414519 U.S. EPA/OPP/ HED 1997b, 2009a
Rabbit	Spinosad (44% a.i. formulation)	Slight transient erythema and edema. Category IV	MRID 43414513 U.S. EPA/OPP/ HED 1997b
Skin Sensitization			
Guinea pig	Spinosad (TGAI, 88-90.4%)	No sensitization.	MRID 43414520 U.S. EPA/OPP/ HED 1997b, 2009a
Guinea pig	Spinosad (44% a.i. formulation)	No sensitization.	MRID 43414514 U.S. EPA/OPP/ HED 1997b

[1] See Section 3.1.11.1 for a discussion of human studies relating to skin irritation in the use of spinosad in the treatment of head lice.

Table A1-5: Eye Irritation Studies

Species	Exposure	Response	Reference
Rabbit	Spinosad	Not an eye irritant (HED 2009a). Slight· conjunctival irritation (HED 1997b). Category IV	MRID 43414518 U.S. EPA/OPP/ HED 2009a
Rabbit	Spinosad (44% a.i. formulation)	Slight conjunctival irritation. Category IV	MRID 43414512 U.S. EPA/OPP/ HED 1997b

[1] See Section 3.1.11.3 for a discussion of human studies relating to eye irritation in the use of spinosad in the treatment of head lice.

Table A1-6: Acute and Repeated Dose Dermal Toxicity

Species	Exposure	Response	Reference
Acute			
Rabbit	Spinosad (TGAI, 88-90.4%)	LD_{50}: >2000 mg/kg bw Category III	MRID 43414516 U.S. EPA/OPP/ HED 1997b, 2009a
Rabbit	Spinosad (44% a.i. formulation)	LD_{50}: >2000 mg/kg bw Category III	MRID 43414510 U.S. EPA/OPP/ HED 1997b
Rabbits, New Zealand white	Spinosad (96.3 % a.i.; 46.1% spinosyn A and 50.2 % spinosyn D)	LD_{50}: >5000 mg/kg bw	FAO/WHO 2011, citing Stebbins and Brooks 1999a
Repeated Dose			
Rabbit	Spinosad (NOS) Doses: 0, 100, 500, or 1000 mg/kg/day Duration: 28 days	NOAEL: 1000 mg/kg/day LOAEL: Not determined.	MRID 43414516 U.S. EPA/OPP/ HED 2009a
Rabbit	Spinosad (XDE-105, 88-90.4%) Doses: 0, 100, 500, or 1000 mg/kg/day Duration: 21 days	NOAEL: 1000 mg/kg/day LOAEL: Not determined.	MRID 43557503 U.S. EPA/OPP/ HED 1997b
Rabbits, New Zealand white, 4 per sex per dose	Spinosad (88%) Doses: 0 or 1000 mg/kg bw/day. Duration: 6 hours/day for 21 days.	NOAEL: 1000 mg/kg/day Includes visual examination of stomach and intestines as well as microscopic examination of ileum, jejunum, colon, and stomach.	FAO/WHO 2001 Cited as Wright et al. 1992b
Rabbits, New Zealand white, 5 per sex per dose	Spinosad (88%) Doses: 0, 100, 500, or 1000 mg/kg bw/day Duration: 6 hours/day for 21 days.	NOAEL: 1000 mg/kg bw/day Includes histopathology of stomach from animals in high dose group.	FAO/WHO 2001 Cited as Vedula and Yano 1994, Study DR-0323-1194-018
Rabbit, New Zealand, 5 per sex per dose.	Spinosad (NAF-85 **formulation**, 43.4%) Doses: 0, 100, 500, or 1000 mg/kg bw/day Duration: 6 hours/day for 21 days Applied as undiluted formulation to the back of each animal and occluded. All animals individually housed.	No mortality or significant treatment-related changes in body weight. Hyperplasia of gastric mucosa at 500 mg/kg bw (4/5 F) and 1000 mg/kg bw (3/5 M and 5/5 F). Increase in aggregates of reticulo-endothelial cells in the dermis at the dermal site occurred in the majority of treated and control rabbits. Higher rate in treated rabbits. Working Note: Responses of 4/5 (p=0.02381) and 5/5 (p=0.003968) are statistically significant using Fischer's Exact test.	Vedula and Yano 1994, Study No. DR-0341-0784-002 and DR-0341-0784-002R Phase 1 Study MRID 43701502 Working Note: Not discussed in EPA risk assessments.

Species	Exposure	Response	Reference
Rabbit, New Zealand, 5 females per dose.	Spinosad (NAF-85 **formulation**, 43.4%) Doses: 0, 200, 300, or 500 mg/kg bw/day Duration: 6 hours/day for 21 days Applied as undiluted formulation. All animals individually housed.	No mortality or significant treatment-related changes in body weight. Hyperplasia of gastric mucosa at 300 mg/kg bw (1/5) and 400 mg/kg bw (5/5). Increase in aggregates of reticulo-endothelial cells in the dermis at the dermal site occurred in the majority of treated and control rabbits. Higher rate in treated rabbits.	Vedula and Yano 1994 Phase 2 Study No. DR-0341-0784-002 and DR-0341-0784-002R MRID 43701502 Working Note: Not discussed in EPA risk assessments.

Table A1-7: Acute Inhalation Toxicity

Source: U.S. EPA/OPP/HED 2004a unless otherwise specified.

Species	Exposure	Response	Reference
Rat	Spinosad (TGAI, 88-90.4%)	LC_{50}: >5.18 mg/L Category IV	MRID 43414517 U.S. EPA/OPP/ HED 1997b, 2009a
Rat	Spinosad (44% a.i. formulation)	LC_{50}: >5 mg/L Category IV	MRID 43414511 U.S. EPA/OPP/ HED 1997b

Appendix 2: Toxicity to birds

Table A2-1: Acute Oral/Gavage Toxicity to Birds

Species	Exposure	Response	Reference[1]
Mallard duck, *Anas platyrhynchos*, 16-weeks-old, 1131 ± 83 g (males), 980 ± 66 g (females), 10/dose group	Spinosad (88% a.i.) for 14 days Doses: 0, 200, 500, 1000, or 2000 mg/kg bw (doses corrected for purity of test substance) Doses administered in three equal amounts over a six hour period.	No mortality or signs of toxicity. LD_{50} >1333 mg/kg bw (2000 mg/kg bw x 0.6666. see comments on multiple dosing) *The authors reported that the study employed triple dosing (three equal doses) over 6 hour period with the second dose given immediately …the EEB considers the highest nominal to be 1333 mg/kg (= 2/3 of the nomina1 2000 mg/kg)* (see p. 5 of DER)	Murray 1992a MRID 43414528 US EPA/OPP/EFED 2011a Supplemental Murray 1992 (DER) Multiple dosing scheme employed in this study constitutes a major study deviation
Northern bobwhite quail, *Colinus virginianus*, 16-weeks-old, 222 ± 17 g (males), 214 ± 15 g (females), 6/dose group	Spinosad (88% a.i.) for 14 days Nominal concentrations: 0, 200, 500, 1000, or 2000 mg/kg bw (doses corrected for purity of test substance)	No mortality. LD_{50} >1333 mg/kg bw. (2000 mg/kg bw x 0.6666. see comments on multiple dosing). NOAEL: 200 mg/kg bw LOAEL: 500 mg/kg bw based on ataxia. 1000 mg/kg bw/day Decreased body weight in females. 2000 mg/kg bw: Decreased body weight in males. *The authors reported that the study employed triple dosing (three equal doses) over 6 hour period with the second dose given immediately …the EEB considers the highest nominal to be 1333 mg/kg (= 2/3 of the nomina1 2000 mg/kg)* (see p. 6 of DER)	Murray et al. 1992b MRID 43414529 US EPA/OPP/EFED 2011a Supplemental Multiple dosing scheme employed in this study constitutes a major study deviation

Table A2-2: Acute Dietary Toxicity to Birds

Species	Exposure	Response	Reference[1]
Mallard duck, *Anas platyrhynchos*, 10-days-old, mean body weight: 148.2 ± 23.9 g, 10/dose group	Spinosad (88% a.i.) for 5 days in the diet, followed by 3 days with untreated food Nominal concentrations: 0, 75, 150, 300, 1250, 2500, or 5000 ppm a.i. (doses adjusted for purity of test substance) Measured concentrations: 76.4, 151, 302, 1243, 2566, or 5156 ppm	No mortality or toxic effects LD_{50} >5156 ppm [≈824 mg/kg bw[1]]	Murray and Woolwine 1992 MRID 43414530 US EPA/OPP/EFED 2011a Acceptable
Northern bobwhite quail, *Colinus virginianus*, 13-days-old, mean body weight: 30.4 ± 2.7 g, 10/dose group	Spinosad (88% a.i.) for 5 days in the diet, followed by 3 days with untreated food Nominal concentrations: 200, 625, 1250, 2500, or 5000 ppm a.i. (doses adjusted for purity of test substance) Measured concentrations: 210, 656, 1335, 2601, or 5253 ppm	One mortality in each of the two highest treatment levels; Loose feces observed in 5253 ppm a.i. treatment group. Mean body weight was significantly reduced in birds fed ≥1335 ppm; during 3-day post treatment observation period, there was no significant reduction in body weight gain at dietary levels of ≤1335 ppm LD_{50} >5156 ppm NOEC = 656 ppm (based on significant body weight reduction at ≥1335 ppm) Approximate NOAEL: 200 mg/kg bw [656 x 0.3 = 196.8 mg/kg bw[1]] Working Note: The estimated NOAEL of ≈200 mg/kg bw is identical to the NOAEL in quail from the gavage study (see Murray et al. 1992b above)	Murray et al. 1992a MRID 43414531 US EPA/OPP/EFED 2011a Acceptable **Note: Use approximate NOAEL of 200 mg/kg bw for acute risk characterization.**

[1]As indicated in a previous Forest Service risk assessment for which both body weights and food consumption rates in acute dietary studies were available for quail and mallards (SERA 2007b), approximate food consumption rates in acute dietary studies are about 0.4 kg food/kg bw for mallards and 0.3 kg food/kg bw for quail. These food consumption rates are from standard studies using very young birds.

Table A2-3: Reproductive Toxicity in Birds

Species	Exposure	Response	Reference[1]
Reproduction			
Northern bobwhite quail, *Colinus virginianus*, 25-weeks-old, 1 male and 1 female/pen, 16 pens/group	Spinosad (88% a.i.) in diet for 21 weeks (1 generation) Nominal concentrations: 0, (acetone) 550, 1100, or 2200 ppm; corn oil vehicle <2% of diet	At 550 ppm: No apparent chronic or reproductive effects observed. At 1100 ppm: A statistically significant reduction live 3-week embryos, normal hatchlings, 14-day-old survivors and hatchling weight, and a reduction in eggs laid (viewed as biologically significant); one mortality and associated necropsy findings similar to the 2200 ppm treatment group. At 2200 ppm: A statistically significant reduction in eggs laid, viable embryos, live 3-week embryos, normal hatchlings, 14-day-old survivors, hatchling weight, 14-day survivor weight and adult terminal body weight; 6 adult mortalities; effects on reproduction condition (regressed ovaries/testes) and gastro-intestinal tract (distended and flaccid livers). NOAEC = 550 mg a.i./kg diet [38.5 mg/kg bw [1]] LOAEC = 1100 mg a.i./kg diet	Beavers et al. 1994a MRID 43414533 US EPA/OPP/EFED 2011a Acceptable
Mallard duck, *Anas platyrhynchos*, 26-weeks-old, 1 male and 1 female/pen, 16 pens/group	Spinosad (88% a.i.) in diet for 20 weeks (1-generation) Nominal concentrations: 0, (acetone) 550, 1100, or 2200 ppm; corn oil vehicle <2% of diet	At 550 ppm: No apparent chronic or reproductive effects observed. At 1100 ppm: A statistically significant reduction in eggshell thickness, eggs laid, viable embryos, live 3-week embryos, normal hatchlings, 14-day-old survivors, terminal female body weight, and increased number of hens with regressing or regressed ovaries and drakes with regressing testes. At 2200 ppm: A statistically significant reduction in eggshell thickness, eggs laid, viable embryos, live 3-week embryos, normal hatchlings, 14-day-old survivors, terminal male and female body weight, and an increased number of hens with regressing or regressed ovaries and drakes with regressing testes. NOAEC = 550 mg a.i./kg diet Approximate NOAEL: 38.5 mg/kg bw. [550 mg/kg food x 0.07 kg food/kg bw = 38.5 mg/kg bw [1]] LOAEC = 1100 mg a.i./kg diet	Beavers et al. 1994b MRID 43414532 US EPA/OPP/EFED 2011a Acceptable

[1] Dietary concentrations (ppm) converted to mg/kg bw doses using food consumption rates of 0.07 kg food/kg bw for reproduction studies in quail and mallards taken from SERA (2007b).

Appendix 3: Toxicity to Terrestrial Invertebrates.

Notes:
NAF-85 refers to Tracer formulation, 44.2% a.i.. See Table 1 of risk assessment.
Unless otherwise specified, references to tables and figures refer to items in the referenced source.
Unless otherwise specified, all toxicity values in units of a.i.

Table A3-1: Honeybees, Toxicity Values

Species	Exposure	Response	Reference
Oral	**Technical Grade**		
Honey bee, *Apis mellifera*	Spinosad (NOS) in acute oral toxicity test	LD_{50} = 0.057 µg/bee Duration not specified.	European Commission 2006
Honey bee, *Apis mellifera* (foragers)	Sweet corn pollen from corn treated at either 70 g/ha (2002 study) or 40 g/ha (2003 study)	No mortality. Food consumption not provided. Pollen from 2003 had residues of 0.32 mg/kg.	Bailey et al. 2005
Honey bee, *Apis mellifera*, NOS	Spinosad (technical grade)	LD_{50}s: 0.06 mg/bee Working Note: See Table 5 of paper. Values are reported as "mg ai/bee". This appears to be a typo. The correct units appear to be µg/bee. See entry from Miles et al. 2002, below. The 0.06 mg a.i./bee is not included in analysis of sensitivities.	Cleveland et al. 2002b
Honey bee, *Apis mellifera*, NOS	Spinosad, technical grade.	48 hour-LD_{50}: 0.063 µg/bee 0.492 µg/g (mg/kg) based on reported body weight of 128 mg.	Mayer et al. 2001
Honey bee, *Apis mellifera*, NOS	Spinosad (technical grade)	24-h LD_{50}: 0.06 µg/bee	Miles et al. 2002
Honey bee, *Apis mellifera*, NOS	Spinosad technical (88% a.i.) In sucrose	48 h-LD_{50}: 0.053 µg/bee Working Note: This is explicitly identified as technical grade. Appears to be a different study the formulation study, summarized below from Mayes et al. 2003 citing Halsall and Grey 1998b.	Mayes et al. 2003 citing unpublished report by Halsall and Grey 1998a. Also in Miles 2003

Species	Exposure	Response	Reference
Oral	Formulation		
Honey bee, *Apis mellifera*	Spinosad (NAF-85) in acute oral toxicity test	LD_{50} = 0.049 µg/bee	European Commission 2006
Honey bee, *Apis mellifera*, NOS	480 g a.i./L SC formulation In sucrose solution	48 hour-LD_{50}: 0.11 µg formulation/L (0.053 µg/bee in Miles 2003 and 0.0528 in Miles et al. 2002)	Mayes et al. 2003 citing Halsall and Grey 1998b Also in Miles 2003 and Miles et al. 2002
Honey bee, *Apis mellifera*, adult workers (Egyptian strain)	Tracer 24% SC Sugar solutions in cotton. Concentrations: 2.5, 5, 10, and 20 mg/L	24 h-LC_{50}: 7.34 mg/L Not clear if this is reported as formulation or a.i. 13.33% mortality at 2.5 mg/L (Table 1). Concentration related inhibition of AChE and ATPase in head, thorax, and abdomen.	Rabea et al. 2010
Contact (µg/bee)	Technical Grade		
Honey bee, *Apis mellifera*, 1- to 4-days-old, 50/treatment group, 2 replicates	Spinosad (88% a.i.) for 48 hours in acute contact study 2 µL acetone applied to thorax and or abdomen of each bee; test doses administered topically in a droplet to thorax and or abdomen of each nitrogen immobilized bee Nominal concentrations: 0, (acetone), 0.0008, 0.0016, 0.0031, 0.0063, or 0.0125 µg a.i./bee	48-h LD_{50} = 0.0029 µg a.i./bee 95% CI = 0.0016-030031µg a.i./bee NOEC = 0.0016 µg a.i./bee, based on treatment-related mortality and signs of toxicity (NOS) at doses ≥0.0031 µg a.i./bee. Taking 116 mg as an average body weight, the NOAEC of 0.0016 µg/bee corresponds to a dose of about 0.014 µg/g bw [0.0016 µg ÷ 0.116 g ≈ 0.01379 µg/g bw (mg/kg bw)] EPA Classification: *Highly toxic to honey bees*	Hoxter et al. 1992 (DER) US EPA/OPP/EFED 2011a MRID 43414547 Acceptable Reported in Mayes et al. 2003 as 48 h-LD50: 0.0024 µg/bee.
Honey bee, *Apis mellifera*, workers	Spinosad (88% a.i.)	LD_{50} = 0.0025 µg a.i./bee `Working Note: This is very similar to Hoxter et al. 1992. Treat are duplicate rather than a new study.`	Miles 2003 (specific study not cited).
Honey bee, *Apis mellifera*, NOS	Spinosad (NOS) in acute contact toxicity test	LD_{50} = 0.0036 µg/bee	European Commission 2006
Honey bee, *Apis mellifera*, workers, from experimental apiary, France. No indication of prior pesticide exposure	Spinosad (99% a.i.) in acetone. Study used acetone and untreated controls.	48 hour LD_{50} = 47.11 ng/bee Dose-related depression in AChE activity (Figure 1).	Carvalho et al. 2013

Species	Exposure	Response	Reference
Honey bee, *Apis mellifera*, NOS	Spinosad (technical grade)	LD_{50}s: 0.0025 mg a.i./bee 0.045 mg/bee No comments on variability in the assays. Working Note: See Table 5 of paper. Values are reported as "mg ai/bee". This may be a typo. The correct units may be µg/bee. These data are not used in analysis.	Cleveland et al. 2002b
Honey bee, *Apis mellifera*, Mean body weight = 127.4 mg	Spinosad (technical grade) Micro-syringe topical application to thorax in acetone.	24 h-LD_{50}: 0.078 µg/bee 0.612 (0.312-0.912) mg/kg bw based on average body weight. See Table 1 of paper. See matched assays on *Megachile rotundata* and *Nomia melanderi* in Table A3-2 below.	Mayer et al. 2001
Honey bee, *Apis mellifera*	Spinosad (technical 88% a.i.)	48 h-LD_{50}: 0.04 µg/bee	Mayes et al. 2003 citing unpublished report by Halsall and Grey l 998a. Also in Miles 2003 review.
Contact (µg/bee)	**Formulations**		
Honey bee, *Apis mellifera*	480 g a.i./L SC	48 h-LD_{50}: 0.12 µg formulation/bee Working Note: ≈0.06 µg a.i./bee	Mayes et al. 2003 citing unpublished report by Halsall and Grey l 998b.
Honey bee, *Apis mellifera*	480 g a.i./L SC	24 h-LD_{50}: 0.88 µg a.i./bee	Miles 2003 and Miles et al. 2002 reviews. Specific study not cited.
Honey bee, *Apis mellifera*	480 g a.i./L SC	48 h-LD_{50}: 1.843 µg formulation/bee Working Note: ≈0.9 µg/bee Working Note: This appears to be the same as the above	Mayes et al. 2003 citing unpublished report by Perina 1996.
Honey bee, *Apis mellifera*	0.2 g a.i./L fruit fly bait	48 h-LD_{50}: >100 µg formulation/bee	Mayes et al. 2003 citing unpublished report by Hahne 2000.
Honey bee, *Apis mellifera*	1.6% WP formulation	24-h LD_{50}: 0.05 µg a.i./bee	Miles et al. 2002

Species	Exposure	Response	Reference
Contact (spray)			
Honey bee, *Apis mellifera* (foragers)	Spinosad (95% a.i.) Direct spray	24-hour LC_{50}: 22 (18-25) mg/L Reported as 2.2 % (w/v) x 10^{-3} Working Note: Compare to other bee species from paper by Scott-Dupree et al. (2009). See Table A3-2 below.	Bailey et al. 2005
Residual contact			
Honey bee, *Apis mellifera* (foragers)	Spinosad (95% a.i.) Corn tassels following treatment at 70 g/ha. Residue of 0.27 mg/kg.	No significant increase in mortality	Bailey et al. 2005
Other			
Honey bee, *Apis mellifera* (NOS)	Spinosad, (TEP: typical end-use product), 23.5% a.i., at a single application rate of 0.16 lb/acre This is a laboratory test designed to determine the length of time over which field-weathered foliar residues (residues on leaves) remain toxic to honey bees. This may be identical to Mayes et al. 2003 citing unpublished studies by Kransfelder 1999; Palmer and Krueger 1997. See Table A3-4 for details.	24-hour RT_{25} = 3 hours (mortality) Working Note: RT_{25} is the residual time required to reduce the activity of spinosad and elicit 25% mortality in caged bees exposed to field-weathered spray deposits.	US EPA/OPP/EFED 2011a MRID 45007701 Acceptable
Honey bee, *Apis mellifera* (NOS)	Spinosad (24% a.i.), no other exposure conditions specified. This is cited in the EFED 2011a document as a *field investigation*. This may be identical to Mayes et al. 2003 citing unpublished study by Mayer 1999. See Table A3-4 for details.	24-hour RT_{25} = 3 hours (mortality)	US EPA/OPP/EFED 2009a MRID 45007702 Supplemental

Table A3-2: Other Bees, Toxicity Values

Species	Exposure	Response	Reference
Acute Oral	**Formulations**		
Bumblebee, *Bombus terrestris*, 4 replicates of 5 workers per replicate in microcolony. [Apidae]	Spinosad, Tracer 480 g/L formulation. Formulation in sugar water. Exposure period: 72 hours.	LC_{50}s: 80 mg a.i./L (without foraging) 44 mg a.i./L (with foraging) See Table 3 of paper which expresses results as fraction of recommended field concentration of 400 mg/L (Table 1 of paper)	Besard et al. 2011
Bumblebee, *Bombus terrestris* [Apidae]	480 g a.i./L SC in sucrose	48-h LC_{50}: 0.0385 µg formulation/bee Approximately 0.018 µg a.i./bee	Mayes et al. 2003 citing unpublished report by Aldershof 1999b.
Stingless bee, *Melipona quadrifasciata* [Apidae]	480 g a.i./L SC (Brazil) in sucrose 24 hour exposure	24 h-LD_{50}: 12.07 ng a.i./bee Working Note: Body weights not specified. Approximate body weight of 80 mg based on average of two other *Melipona* species from Thompson 2015. Based on this assumption, LD50 ≈ 0.15 mg/kg bw. Sublethal effects included impaired flight. Reduced respiratory rate at 24 hours after exposure (Figure 4) but not significant with respect to controls.	Tom et al. 2015

Species	Exposure	Response	Reference
Longer-term Oral	**Formulations**		
Stingless bee, *Melipona quadrifasciata* [Apidae], 96 larvae per dose. Average weight: 96.80±0.97 mg	480 g a.i./L SC (Brazil) in diet 20 day exposure period, approximately the entire larval feeding period. Doses as reported: 0.57, 1.14, 2.29, 11.4, 22.9, 114, 228, 1142, and 11424 ng a.i./bee. Cumulative doses in mg/kg bw: 0.0059, 0.012, 0.024, 0.12, 0.24, 1.18, 2.36, 11.8, 118 mg/kg bw. Average daily doses in mg/kg bw: 0.00029, 0.00059, 0.0012, 0.0059, 0.012, 0.059, 0.12, 0.59, 5.9 mg a.i./kg bw/day.	Reported toxicity values for larvae: NOAEL: 22.9 ng a.i./bee [0.012 mg a.i./kg bw/day] LOAEL: 114 ng a.i./bee [0.059 mg a.i./kg bw/day] based on decreased survival, decreased pupal body weights, and increase in malformations (larvae and adults). 50% mortality at about 114 t0 228 ng/bee (≈0.056 to 0.12 mg/kg bw/day) estimated from Figure 1B of paper. Walking behavior in newly emerged adults: NOAEL: 1.14 ng a.i./bee [0.00059 mg/kg bw/day] LOEAL: 2.29 ng a.i./bee [0.0012 mg/kg bw/day], See Figure 6 of paper for details. Mortality in all bees at two highest doses prior to emergence.	Barbosa et al. 2015 Working Note: The paper does not explicitly state that the doses reported in the paper are cumulative. The corresponding author was queried and confirmed that the doses were cumulative. Thus, the average daily doses in column 2 are calculated as the cumulative doses divided by 20 days.
Acute Contact			
Alfalfa leafcutter bee, *Megachile rotundata* [Megachilidae] Mean body weight = 30.4 mg	Spinosad (technical grade) Micro-syringe topical application to thorax in acetone.	LD_{50} 0.058 µg a.i./bee 1.908 (0.461-2.51) mg/kg bw based on average body weight. See Table 1 of paper. See matched assays on *Apis mellifera* (Table A3-1) and *Nomia melanderi* (this table).	Mayer et al. 2001
Alkali bee, *Nomia melander* [Halictidae] Mean body weight = 85.2 mg	Spinosad (technical grade) Micro-syringe topical application to thorax in acetone.	LD_{50}: 0.065 µg a.i./bee 0.763 (0.553-0.973) mg/kg bw based on average body weight. See Table 1 of paper. See matched assays on *Apis mellifera* (Table A3-1) and *Megachile rotundata* (this table).	Mayer et al. 2001
Bumblebee, *Bombus terrestris* [Apidae]	Spinosad, Tracer 480 g/L formulation	48-h LD_{50}: 19.4 µg a.i./bee 72-h LD_{50}: 15.5 µg a.i./bee Working Note: The units are µg a.i./bee. This study is not in ECOTOX (https://cfpub.epa.gov/ecotox/).	Mayes et al. 2003 citing unpublished report by Aldershof 1999a.

Species	Exposure	Response	Reference
Bumblebee, *Bombus terrestris* [Apidae]	Spinosad, 480 g a.i./L formulation	Wet residue 72 h-LC_{50}: 0.085 mg/L Dry residue 72 h-LC_{50}: 2.4 mg/L	Besard et al. 2011
Contact (spray)			
Bumblebee, *Bombus impatiens* [Apidae]	Spinosad (90% a.i.) 4-6 replicates of 9-11 bees per concentration.	48 h LC_{50}: 89.5 (79.2-100.6) mg/L Reported as 8.95% $x10^{-3}$ w/v Compare to honey bees, Bailey et al. 2005	Scott-Dupree et al. 2009
Alfalfa leafcutting bee, *Megachile rotundata* [Megachilidae]	Spinosad (90% a.i.) 4-6 replicates of 9-11 bees per concentration.	48 h LC_{50}: 12.5 (11.3-14) mg/L Reported as 1.25% $x10^{-3}$ w/v Compare to honey bees, Bailey et al. 2005	Scott-Dupree et al. 2009
Blue orchard bee, *Osmia lignaria* [Megachilidae]	Spinosad (90% a.i.) 4-6 replicates of 9-11 bees per concentration.	48 h LC_{50}: 47 (40-54) mg/L Reported as 4.7% $x10^{-3}$ w/v Compare to honey bees, Bailey et al. 2005	Scott-Dupree et al. 2009
Longer-term	**spray**		
Bumblebee, *Bombus terrestris*, 4 replicates of 5 workers per replicate in microcolony.	Spinosad, Tracer 480 g/L formulation. Formulation in sugar water. Exposure period: 11 week with solutions refreshed weekly. No foraging.	LC_{50}: 1.6 mg a.i./L NOEC: 0.4 mg a.i./L LOAEC: 4 mg a.i./L based on reduction in nest reproduction (due to worker mortality) See Table 3 of paper which expresses results as fraction of recommended field concentration of 400 mg/L (Table 1 of paper)	Besard et al. 2011[1]
Bumblebee, *Bombus terrestris*, 4 replicates of 5 workers per replicate in microcolony.	Spinosad, Tracer 480 g/L formulation. Formulation in sugar water. Exposure period: 7 weeks with solutions refreshed weekly. Bees allowed to forage.	LC_{50}s: 3.9 mg a.i./L NOEC: 0.4 mg a.i./L LOAEC: 4 mg a.i./L based on nest reproduction and the numbers of drones produced. See Table 3 of paper which expresses results as fraction of recommended field concentration of 400 mg/L (Table 1 of paper).	Besard et al. 2011[1]

[1] Basard et al. 2011 state in the abstract: *Another important conclusion is that the present data provide strong evidence that neither spinosyn has a negative effect on the foraging behaviour of these beneficial insects*. This generalization appears to be inconsistent with their data at LOAELs and above.

Table A3-3: Other Arthropods, Toxicity Values

Species	Exposure	Response	Reference
Acute Oral			
Bactrocera dorsalis, oriental fruit fly [Diptera: Tephritidae]	Spinosad (technical grade)	24-h LC_{50}: 30.3 µg/mL for sensitive strain Resistance Factor up to 782 (Table 5).	Hsu and Fend 2006
Acute Injection			
Heliothis virescens (larvae) [Lepidoptera : Noctuidae]	Spinosyn A	24-h LD_{50}: 0.014 (0.006–0.031) µg/larva	Salgado 1998
Periplaneta americana, adults [Blattodea: Blattidae]	Spinosyn A	24-h LD_{50}: 0.74 (0.41–1.34) µg/animal	Salgado 1998
Periplaneta americana, adults [Blattodea: Blattidae]	Spinosyn A	24-h LD50: 1.9 µg/animal	Salgado et al. 1998
Acute Contact			
Aedes aegypti, [Diptera: Culicidae]	Spinosad (technical grade)	24-h LD_{50}: $8.9x10^{-4}$ ($7.7x10^{-4}$ to $1.1x10^{-3}$) µg/mg bw (Table 2)	Pridgeon et al. 2008
Culex quinquefasciatus [Diptera: Culicidae]	Spinosad (technical grade)	24-h LD_{50}: $3.2x10^{-3}$ ($2.3x10^{-3}$ to $5x10^{-3}$) µg/mg bw (Table 3)	Pridgeon et al. 2008
Anopheles quadrimaculatus [Diptera: Culicidae]	Spinosad (technical grade)	24-h LD_{50}: $1.5x10^{-3}$ ($1.2x10^{-3}$ to $1.9x10^{-3}$) µg/mg bw (Table 4)	Pridgeon et al. 2008
Musca domestica [Diptera: Muscidae]	Spinosad (NOS)	72-h LD_{50}: 24.2 (19.2-29.2) ng/fly Resistance factors up to 4.3 (Table 1). Some wild strains more sensitive than laboratory strain.	Scott 1998
Musca domestica [Diptera: Muscidae]	Spinosad (93%) 3 replicates of each bioassay.	72-h LD_{50}: 0.74 (0.59-0.9) ng/fly Resistance factor up to 279 over 27 generations of rearing. Selection pressure of 60-80% lethal doses for cultured generations.	Shi et al. 2011
Bactrocera dorsalis, oriental fruit fly [Diptera: Tephritidae]	Spinosad (technical grade)	24-h LD_{50}: 59.6 (49.8-71.3) ng/fly for sensitive strain Resistance Factor >480 (Table 3).	Hsu and Fend 2006
Bactrocera dorsalis, oriental fruit fly [Diptera: Tephritidae]	Spinosad (technical grade)	24-h LD_{50}: 40.9 (33.6-49.2) ng/fly for sensitive strain Resistance Factor > 2445 (Table 2).	Hsu et al. 2012b
Bactrocera cucurbitae, melon fly [Diptera: Tephritidae]	Spinosad (Success SC 22.8% a.i.)	<table><tr><th>Duration (hours)</th><th>LD_{50} ng/fly</th></tr><tr><td>24</td><td>5.0</td></tr><tr><td>48</td><td>3.16</td></tr><tr><td>72</td><td>3.07</td></tr></table> Above are for laboratory strain. Resistance factors of up to about 5 (Table 2)	Hsu et al. 2012a

<table>
<tr><th>Species</th><th>Exposure</th><th>Response</th><th>Reference</th></tr>
<tr><td>Manduca sexta,
tobacco budworm
[Lepidoptera:
Sphingidae],
Larvae, 4 to 5 days old (second instar), 20 to 40 mg bw. 30-40 larvae in 6 to 8 replicates per dose.</td><td>Spinosad</td><td><table><tr><th>Duration (hours)</th><th>LD_{50} ng/larva</th><th>LD_{50} mg/kg</th></tr><tr><td>24</td><td>59</td><td>1.97</td></tr><tr><td>48</td><td>2</td><td>0.067</td></tr><tr><td>72</td><td>0.4</td><td>0.013</td></tr></table>Use average body weight of 30 mg to estimate mg/kg bw dose.
See Table 3 and Figure 3 of paper.</td><td>Herzog et al. 2002</td></tr>
<tr><td>Helicoverpa armigeram, cotton bollworm, late second or early third instar, 8–15 mg,
[Lepidoptera:
Noctuidae]
5 strains</td><td>Spinosad (NOS)
Five concentrations, at least 24 larvae per dose.</td><td>48-h LD_{50}s: Average: 1.6 ng a.i./mg with range of 0.8 to 2.6 ng a.i./mg.
See Table 3 of paper for details.

Factor of 3.35 in variability appears to reflect simple variation in strains from different locations.

A strain resistant to pyrethroids evidenced no cross-resistance to spinosad.</td><td>Achaleke et al. 2009</td></tr>
<tr><td>Hyposoter didymator,
[Hymenoptera:
Ichneumonidae]
lepidopteran parasitoid</td><td>Tracer48 SC</td><td>48-h LD_{50}: 0.5 µg/g or ng/mg pupa
Working Note: See Table 2 for exposures in mg a.i./L with dose to pupae in µg/g in Table 1. LD_{50} based on 50% mortality in adults after emergence.</td><td>Schneider et al. 2003</td></tr>
</table>

Table A3-4: Field or Field Simulation Studies in Bees

Note: This table is organized by study type (greenhouse/field simulation/field stud) and within each study type the studies are listed roughly by application rate.

Species	Exposure	Response	Reference
Greenhouse			
Honeybees (NOS)	Formulation: 250 g a.i./L SC formulation Application Rate: 100 g a.i./ha [0.089 lb a.i./acre] to strawberries.	Inhibition of larval growth at 1 and 3 days after application	Mayes et al. 2003 citing unpublished studies by Kaneshi 2000b
Bumblebees (NOS)	Formulation: 250 g a.i./kg WDG Application Rate: 120 a.i./ha [≈0.11 lb a.i./acre]	No effect on foraging. Reduction in number of adult bees returning on Day 0 and Day 2. Inhibition of larval growth at Days 0, 2 and 4 after application. No inhibition by Day 8.	Mayes et al. 2003 citing unpublished studies by Kaneshi 2000a. Kaneshi study also briefly noted in Morandin et al. 2005
Bumble bees (NOS)	Formulation: 480 g a.i./L SC Application Rate: 540 g a.i./ha [≈0.48 lb a.i./acre] applied to tomato plants	Temporal (NOS) effects on foraging. Slight reduction (NOS) in brood development.	Mayes et al. 2003 citing unpublished studies by Aldershoff 2000
Field Simulation			
Honeybees, *Apis mellifera*	Brief summary of studies conducted between 2002 to 2010 at rates of 96 to 144 g a.i./ha [0.086 to 0.13 lb a.i./acre].	No substantial impacts (few details).	Miles et al. 2011 Review of unpublished studies. Not as specific as Mayes et al. 2003.
Honeybees, *Apis mellifera*, 4 replicates, 30 bees per replicate Alfalfa leafcutter bee, *Megachile rotundata*, 4 replicates, 20 bees per replicate. Alkali bee, *Nomia melander*, 4 replicates, 20 bees per replicate.	Three formulations: 1.6% WP, 80WDG, and 2SC at application rates of 0.05 to 0.2 kg a.i./ha.. The 2SC formulation also applied at 0.1 lb a.i. with or without adjuvants. Assays with vegetation collected at 2 and 8 hours after application.	Honeybees No mortality in excess of 4%. No substantial difference between 2 and 8 hour post-application assays. Leafcutter bees Mortality rates of 5 to 31%. Lower mortality at 8 relative to 2 hours in 8/12 comparisons Alkali bees Mortality rates of 2 to 29%. Lower mortality at 8 relative to 2 hours in 11/12 comparisons Working Note: See Table 3 of paper for details.	Mayer et al. 2001

Species	Exposure	Response	Reference
Honeybees, *Apis mellifera*	Formulation: 240 g a.i./L SC Application Rate: 42 g a.i./ha or 177 g a.i./ha [≈0.04 lb a.i./acre or 0.16 lb a.i./acre] Laboratory simulation. Treated alfalfa plots with exposures 3 hours after application.	No signs of toxicity.	Mayes et al. 2003 citing unpublished studies by Kransfelder 1999; Palmer and Krueger 1997.
Honeybees, hives	Formulation: 480 g a.i./L SC Application Rate: 216 g a.i./ha [0.20 lb a.i./acre] applied to tansy phacelia Four applications on study days 0, 7, 17, and 9. Fourth application made when bees were active.	Transient effects on foraging (on 4 of 18 observations. No reduction in brood development.	Mayes et al. 2003 citing unpublished study by Halsall 2002. Also in Miles 2003 (see Figures 4, 5 and 6)
Honeybees, hives confined in 4x4.5 m area.	Formulation: 480 g a.i./L SC Application Rate: 144 or 540 g a.i./ha [0.13 or 0.48 lb a.i./acre] applied to tansy phacelia. Applications in morning prior to bee activity. Observations at 7 days after treatment.	Reduced number of foraging bees at higher application rate. Slight decline at lower rate. Slight increase in mortality on Day 1 after treatment at the higher application rate but this does not appear to be statistically significant (Figure 3). Reduction in brood development at higher application rate. No effect at lower rate.	Mayes et al. 2003 citing unpublished study by Vinall 2000 Also in Miles 2003 (see Figures 1-3)

Species	Exposure	Response	Reference
Bumblebee (*Bombus impatiens*) colonies of 5-10 bees/colony. 7 colonies per dose group (including controls).	Spinosad (90.4%) Bees fed pollen at 0 (acetone control), 0.2, 0.8 and 8 mg/kg pollen (consumed by larvae). Also fed untreated sucrose solution. Study Duration: 10 weeks Exposures: Weeks 3 to 5 of study. Foraging on artificial flowers Working Note: See discussion of application rates relative to residues on p. 3 of paper. 0.2 mg/kg = 20 g/ha [0.017 lb a.i./acre] 0.8 mg/kg ≈ 80 g/ha [0.07 lb a.i./acre] 8.0 mg/kg ≈ 800 g/ha [0.71 lb a.i./acre] Above are just crude approximations.	0.2 mg/kg Group: No effects on colony health or foraging. 0.8 mg/kg Group: No effects on overall colony health but lower worker larvae weights. During foraging, an increase in handling times (slower bees) relative to controls and low dose groups. Signs of trembling during foraging. 8 mg/kg Group: Decline in colony health by Weeks 4 or 5. Decline in number of workers by week 5. Bee in most colonies dead by Week 10. Decrease in body weights of workers starting in Week 5. Decrease in amount of brood starting at Week 6 (Figure 4). Progression from larvae to pupae arrested in Week 4. Foraging not evaluated due to high mortality. No signs of avoidance of treated flowers	Morandin et al. 2005 Primary literature. Simon Fraser University, Canada
Stingless bee (*Plebeia moureana*), 40 individuals trained for foraging experiment.	GF-120 Formulation (used for fruit fly control. Feeding in sucrose solutions. Concentrations: 0, 10, 20, 40, and 80 mg a.i./L. Working Note: 80 mg a.i./L considered 'worst case' for field exposures. Duration of test: 35±15 minutes.	No avoidance. No effect on foraging behavior. Working Note: Authors discuss that the short-term observations may have been inadequate to assess longer-term effects on foraging activity.	Sanchez et al. 2012 Primary literature. Mexico. Public research center.
Field Studies			
Honeybees, *Apis mellifera*. 2 hives per treatment block (3.2 ha), 4 blocks.	Formulation: Spinosad (SolBait) Application Rate: up to 1.57 g a.i./ha [0.0014 lb a.i./acre] for the control of fruit flies. Three applications at two week intervals. Observations at 14 days following each application.	No effects on brood numbers (Table 2 of paper) or subjective assessments of colony health (Table 3 of paper).	Burns et al. 2001 Primary literature. USDA in cooperation with Dow AgroSciences.

Species	Exposure	Response	Reference
Honeybees, 5 colonies per plot	Formulation: 240 g a.i./L SC Application Rate: 70 or 175 g a.i./ha [0.062 or 0.16 lb a.i./acre] applied to alfalfa Aerial (helicopter) Observations up to 5 days after treatment. Treated vegetation covered for 3 hours (drying time).	No effects based on mortality or brood development. See Miles 2003 for statistics.	Mayes et al. 2003 citing unpublished study by Mayer 1999. Also in Miles 2003 (see Table 5)
Honeybees, 4 colonies per orchard.	Formulation: 120 g a.i./L SC Application Rate: 96 g a.i./ha [0.086 lb a.i./acre] applied to flowering avocado Evening application which dried prior to bee exposure. 14 treated orchards. Duration of observation not clear.	No significant or substantial impact on brood areas (Figure 7) or brood mortality.	Mayes et al. 2003 citing unpublished study by Taylor and Goodwin 2000.
Honeybees	Formulation: 240 g a.i./L SC Application Rate: 100 g a.i./ha (0.089 lb a.i./acre) to almond trees in the night. Ground (orchard sprayer) application. Two 3.6 ha plots with one 4.5 ha control plot Observations for 12 days.	No significant effects noted on mortality, brood development, or foraging.	Mayes et al. 2003 citing unpublished study by Forey 1999.
Honeybees, 19 to 20 bees per group.	Formulation: 120 g a.i./L SC Application Rate: 96 or 192 g a.i./ha [0.086 or 0.17 lb a.i./acre] applied to kiwi Morning or evening applications by hand-held spray gun to groups of 10 vines. 72 hour observation of captured bees.	Very slight but apparently dose-related increase in mortality in captured bees over a 72 hour observation period (Figure 6). Effect does not appear to be statistically significant but statistical analyses are not explicitly discussed. Commentary in Review (p. 62): *These data demonstrate that the evening or early morning application of spinosad at 96 g a.i. or 192 g a.i./ha to kiwifruit does not affect the survival or foraging of honeybees exposed to pollen or nectar.*	Mayes et al. 2003 citing unpublished study by Goodwin and Haine 1998.

Species	Exposure	Response	Reference
Honeybees, two plots, 5 hives/plot, placed 12 hours prior to applications	Formulation: 240 g a.i./L SC Application Rate: 157 g a.i./acre [0.14 lb a.i./acre] applied to citrus at night with orchard sprayer. Observations for 12 days after treatment.	No effects.	Mayes et al. 2003 citing unpublished study by Kirkland 1999.
Honeybees, two plots, 5 hives/plot, placed 12 hours prior to applications	Formulation: 240 g a.i./L SC Application Rate: 210 g a.i./ha [0.19 lb a.i./acre] applied to citrus at night with orchard sprayer. Observations for 12 days after treatment.	Mortality higher on treated plots but not statistically significant (Figure 5). No apparent effects on brood development or foraging.	Mayes et al. 2003 citing unpublished study by Kirkland 1999.
Honeybees, *Apis mellifera*	GF-120: Applications for control of fruit flies. 6 - 18 days Application rate and other details not discussed in EFED document.	No adverse effects at use rate (NOS) Working Note: A study by Rendon is not included in the review by Mayes et al. 2003.	US EPA/OPP/EFED 2011a MRID 45708201/ 45708801 Cited as Rendon et al, No date. Supplemental

Table A3-5: Toxicity to Earthworms

Species	Exposure	Response	Reference
Acute			
Earthworm, *Eisenia foetida*	Spinosad (88% .a.i) for 14 days	14- day LC_{50} >970 mg a.i./kg soil (based on weight decreases) NOAEC = 970 mg a.i/kg soil	US EPA/OPP/EFED 2011a; 2009a MRID 43414548 Supplemental
Earthworm, *Eisenia foetida*	Spinosyn B	LC_{50} >1000 mg/kg soil (correcte4d value: >500 mg/kg soil)	European Commission 2006
Earthworm, *Eisenia foetida*	N-Demethylated spinosyn D	LC_{50} >1000 mg/kg soil (correcte4d value: >500 mg/kg soil)	European Commission 2006
Reproduction			
Earthworm, *Eisenia foetida*	Spinosad, NAF-85	NOEC >2700 g as/ha (corrected value: 1350 g as/ha)	European Commission 2006
Earthworm, *Eisenia foetida*	Spinosyn B	NOEC ≥3.582 mg/kg soil (corrected value: ≥1.791 mg/kg soil)	European Commission 2006
Earthworm, *Eisenia foetida*	N-Demethylated spinosyn D	NOEC ≥1.928 mg/kg soil (corrected value: ≥0.964 mg/kg soil)	European Commission 2006

Appendix 4: Toxicity to Terrestrial Plants

Working Note: The studies on vegetative vigor and seedling emergence are conducted as a rate higher than that proposed by the Forest Service. U.S. EPA/OPP/EFED (2011a, p. 56) has noted that the maximum application rate for spinosad is ≈0.8 lbs a.i./acre and that an …*acceptable tier I study is needed that tests the effects of the maximum labeled application rate to terrestrial plants*.

Table A4-1: Vegetative Vigor

Species	Exposure	Response	Reference [1]
Monocots			
Corn, *Zea mays* (Poaceae) Oat, *Avena sativa*, (Poaceae) Wheat, *Triticum aestivum* (Poaceae) Onion, *Allium cepa* (Liliaceae)	Spinosad, formulated product (44.2% a.i.), 0.5 lb a.i./acre	No phytotoxic or other effects *Based on these results, terrestrial plant risk is considered minimal, and further plant tests (Tier II) are not required*	U.S. EPA/OPP/EFED 2010a MRID 44597732 Acceptable
Dicots			
Carrot, *Daucus carota* (Apiaceae) Cucumber, *Cucumis sativus* (Cucurbitaceae) Radish, *Raphanus sativus* (Brassicaceae) Soybean, *Glycine max* (Fabaceae) Sunflower, *Helianthus annuus* (Asteraceae) Tomato, *Lycopersicon esculentum* (Solanaceae)	Spinosad, formulated product (44.2% a.i.), 0.5 lb a.i./acre	No phytotoxic or other effects Radish shown to be the most sensitive dicot *Based on these results, terrestrial plant risk is considered minimal, and further plant tests (Tier II) are not required*	U.S. EPA/OPP/EFED 2010a MRID 44597732 Acceptable

Table A4-2: Seedling Emergence Vigor

Species	Exposure	Response	Reference [1]
Monocots			
Corn, *Zea mays* (Poaceae) Oat, *Avena sativa*, (Poaceae) Wheat, *Triticum aestivum* (Poaceae) Onion, *Allium cepa* (Liliaceae)	Spinosad, TGAI (88% a.i.), 200 g a.i./hectare (0.18 lb a.i./acre)	No phytotoxic effects greater than 25%, based on shoot weight and shoot length *Based on these results, terrestrial plant risk is considered minimal, and further plant tests (Tier II) are not required*	U.S. EPA/OPP/EFED 2010a MRID 43701506 Acceptable
Dicots			
Carrot, *Daucus carota* (Apiaceae) Cucumber, *Cucumis sativus* (Cucurbitaceae) Radish, *Raphanus sativus* (Brassicaceae) Soybean, *Glycine max* (Fabaceae) Sunflower, *Helianthus annuus* (Asteraceae) Tomato, *Lycopersicon esculentum* (Solanaceae)	Spinosad, TGAI (88% a.i.), 200 g a.i./hectare (0.18 lb a.i./acre)	No phytotoxic effects greater than 25%, based on shoot weight and shoot length Radish shown to be the most sensitive dicot *Based on these results, terrestrial plant risk is considered minimal, and further plant tests (Tier II) are not required*	U.S. EPA/OPP/EFED 2010a MRID 43701506 Acceptable

Appendix 5: Toxicity to fish.

Table A5-1: Acute Toxicity in fish

Species	Exposure	Response	Reference
Technical Grade			
Bluegill sunfish, *Lepomis macrochirus*, approximately11-weeks-old, 0.22-0.68 g, 29-42 mm, 10/concentration level	Spinosad, technical grade (88% a.i.) under static conditions for 96 hours Nominal concentrations: 1.0, 2.5, 5.0, 6.5, 8.0, or 9.5 mg/L Mean measured concentrations: 0, 0.95, 2.10, 4.60, 7.05, 7.30, or 9.05	96-hour LC_{50} = 5.94 mg/L NOEC for mortality: 4.6 mg/L. NOAEC for Signs of Toxicity: 2.1 mg/L. LOAEC: 4.6 mg/L based on labored respiration and hypoactivity. Ratio of NOAEC to LC_{50}: 2.1 ÷ 5.94 ≈ 3.5. Working Note: The lower ratio of 0.23 from York (1993) is used to estimate NOAECs when needed.	Newsted and Brock 1992 (DER) U.S. EPA/OPP/ EFED 2011a MRID 43414534 Acceptable Cleveland et al. 2002b (DOW ERA)
Carp, *Cyprinus carpio*, juveniles, 10/treatment	Spinosad (NOS),under static conditions Mean measured concentrations: 0., 0.7, 3.4, 4.0, 4.2, 4.5, or 6.0 mg/L	96-hour LC_{50} = 4.99 mg/L Estimated NOAEC: 1.4 mg/L [4.99 mg/L x 0.23 ≈ 1.127] See York 1993 entry for 0.23 factor.	Cleveland et al. 2002b (DOW ERA)
Carp, *Cyprinus carpio*	Spinosad (NOS) for 96 hours	96-hour LC_{50} = 4.0 mg as/L (nominal)	European Commission 2006
Rainbow trout, *Oncorhynchus mykiss*, 10/treatment	Spinosad, technical grade (88% a.i.) under static conditions for 96 hours Mean measured concentrations: 0, 5.3, 7.3, 9.5, 13, 17, 23, 30, or 41 mg/L	96-hour LC_{50} = 30.0 mg/L Estimated NOAEC: 6.9 mg/L [30 mg/L x 0.23]. See York 1993 entry for 0.23 factor.	U.S. EPA/OPP/ EFED 2011a MRID 43444103 Acceptable Cleveland et al. 2002b (DOW ERA)
Sheepshead minnow, *Cyprinodon variegatus*, 0.15-0.48 g at termination, 7-25 mm, 10/treatment chamber	Spinosad, technical grade (87.9% a.i.), 24-hour static renewal for 96 hours Nominal concentrations: 0, 1.6, 2.6, 4.3, 7.2, or 12 mg/L Mean measured concentrations: 1.80, 2.95, 4.87, 7.38, or 10.6 ppm	96-hour LC_{50} = 7.87 ppm 95% CI = 4.87-10.6 ppm NOEC = 1.8 ppm (based on no mortality) Working Note: Neither most nor least sensitive. Use NOAEC ratio of 0.23 [1.8 ÷ 7.87 ≈ 0.2287] to estimate NOAECs for other species.	York 1993 (DER) U.S. EPA/OPP/ EFED 2011a Acceptable

Appendix 5: Toxicity to fish *(continued)*

Species	Exposure	Response	Reference
Formulations			
Carp, *Cyprinus carpio*	NAF -85	96-hour LC_{50} >49 mg a.i./L (nominal)	European Commission 2006
Coho salmon, *O. kisutch*, 6- to 9-months-old, average length: 7.96 ± 0.12 cm, average weight: 5.03 ± 0.28 g	Spinosad (Success), 240 g a.i./L for 96 hours under static non-renewal conditions. Nominal concentrations 1, 10, 50, 100, 250, or 500mg a.i./L	96-hour LC_{50} >500 mg a.i./L [Table 2 of paper] NOAEC for swimming behavior: 10 mg a.i./L [p. 552, column 1 of paper]	Deardorff and Stark 2009 Washington State University
Nile tilapia, *Oreochromis niloticus*, juveniles, 11.55 ± 1.2g, 9.28 ± 0.54 cm, 6 fish/group, 3 replicates	Commercial formulation of spinosad, Laser (480 g/L a.i.) 0, 25, 50, or 75 mg/L in static-renewal system for 24-48-72 hours	Significant ($P<0.05$) inhibition of acetylcholinesterase enzyme activities at all treatment levels and durations in the brain (from 21 to 35%) and in the liver (from 32 to 63%). Observations included erratic swimming, loss of balance, and slow gill movement at 75 mg/L spinosad for 72 hours without mortality. NOAEL for signs of toxicity: 50 mg/L. NOAEL for mortality: 75 mg/L.	Piner and Uner 2012 Turkey
Nile tilapia, *Oreochromis niloticus*, juveniles	Commercial formulation of spinosad, Laser (480 g/L a.i.) 0, 5, 25, 50, 75, 100, 125, or 150 mg/L for 96 hours	Mortality observed at >100 mg/L. NOAEL for mortality: 75 mg/L.	Piner and Uner 2013 Turkey
Nile tilapia, *Oreochromis niloticus*, juveniles	Commercial formulation of spinosad, Laser (480 g/L a.i.) 0, 25, 50, or 75 mg/L in static-renewal system for 24-48-72 hours	Significant effects on glutathione-related oxidative stress markers, lipid peroxidation, heat shock proteins, and apoptosis in the liver. Significant decrease in glutathione 75 mg/L (Table 1). No clear concentration-response for GSH/GSSG ratios (Table 2).	Piner and Uner 2013 Turkey

Appendix 5: Toxicity to fish *(continued)*

Species	Exposure	Response	Reference
Nile tilapia, *Oreochromis niloticus*, juveniles, 11.55 ± 1.2g, 9.28 ± 0.54 cm, 6 fish/group, 3 replicates	Commercial formulation of spinosad, Laser (480 g/L a.i.) 0, 25, 50, or 75 mg/L in static-renewal system for 24-48-72 hours	Observations included significant increases at 24 hours in the levels of tGSH: 22% at 50 mg/L and 33% at 75 mg/L. Treatment also decreased the ratio of GSH/GSSG and GPx activity as well as an induction in the GR activity. The results indicate that spinosad had oxidative effects in the brain tissue by altering the parameters of the GSH-related antioxidant system and the Hsp 70 levels.	Pine and Uner 2014 Turkey
Zebrafish (*Danio rerio*), embryo-larvae, 2-4 replicates of 6 larvae/replicate	SpinTor™ 0, 0.2, 0.75, 2.0, 3.0, 7.5, or 30 ppb for 7 days. Exposure: On day 4, larvae were transferred from exposure dishes to well plates (one larvae/plate) and exposed to either substrate alone or substrate plus phorbol 12-myristate 13-acetate (PMA). In healthy fish PMA evokes respiratory burst response.	No effect on the innate immune system (measured as the respiratory burst response) of embryo-larval zebrafish. Response was measured for 2 hours in fish exposed to the 4 lowest doses and for 3.5 hours in fish exposed to the 2 highest doses	Elskus 2007 USGS and Maine Department of Environmental Protection

Table A5-2: Longer-term toxicity in fish

Species	Exposure	Response	Reference
Mosquito Fish, *Poecilia reticulata*, adults, mean length 3.5 ± 0.2 cm, 21 fish/group Mature fish	Spinosad (NOS) 0, 60, 123, or 361 µg/L in static renewal test. Duration: 28 days Micronucleus assay	Genotoxicity manifested by inhibition of mitotic division, which the authors state could affect growth of the exposed fish. The induction of micronucleus, nuclear abnormal, and normochromatic cells, evaluated from blood samples collected from the gill epithelial cells, was highly significant ($P<0.01$; $P<0.001$). No indication of cytotoxicity. Working Note: The effects were not concentration related (See Figure 1 of paper). No marked temporal effect.	Anogwih et al. 2003 Nigeria
Rainbow trout, *Oncorhynchus mykiss*, NOS Age of fish not specified.	Spinosad, NOS, under flow-through conditions for 21 days <u>Mean measured concentrations</u>: 0, 0.63, 1.2, 2.1, 3.7, 6.0, or 10.2 mg/L	21-day LC_{50} = 4.8 mg/L NOEC = 1.2 mg/L LOAEC = 2.1 mg/L	Cleveland et al. 2002b (DOW ERA) Not summarized in EPA risk assessments.
Rainbow trout, *Oncorhynchus mykiss*, embryos 2- to 24-hours old, 4 replicates, 50 embryos/replicate reduced to 25 embryos/ replicate on Day 17. Early life-stage	Spinosad, technical grade (88% a.i.) in early life stage study under flow-through conditions. <u>Nominal concentrations</u>: 0, 0.25, 0.50, 1.0, 2.0, 4.0, or 8.0 mg/L <u>Mean measured concentrations</u>: 0.251, 0.498, 0.962, 1.89, 3.79, or 7.81 ppm. Duration:	No statistically significant differences observed for % embryos hatched, % normal larvae at hatch, or % survival to thinning. Adverse effects included statistically significant reduction in growth (length and weight) at 3.76 ppm; a statistically significant reduction in survival at 1.89 ppm, and a statistically significant reduction in day to mean hatch at 0.962 ppm. NOAEC = 0.498 ppm LOAEC (hatching) = 0.962 ppm LOAEC (survival) = 1.89 ppm LOAEC (body length) = 3.76 ppm	Weinberg et al. 1993 (DER) U.S.EPA/OPP/ EFED 2011a MRID 43414541 Acceptable Also summarized Cleveland et al. 2002b.

Appendix 5: Toxicity to fish *(continued)*

Species	Exposure	Response	Reference
Sheepshead minnow, *Cyprinodon variegatus*, 40/replicate Early life-stage	Spinosad, technical grade (88% a.i.) for 37 days under flow-through conditions Mean measured concentrations: 0.511, 1.15, 2.38, 4.84, or 9.63 mg/L	NOAEC = 1.15 ppm LOAEC (reduced growth) = 2.38 ppm	US EPA/OPP/EFED 2011a MRID 44420601 Acceptable Also summarized Cleveland et al. 2002b (DOW ERA)
Zebrafish, *Danio rerio*, embryo-larvae, 2-4 replicates of 6 larvae/replicate Early life-stage	SpinTor™ 0, 0.2, 0.75, 2.0, 3.0, 7.5, or 30 ppb for 7 days. Exposure: On day 4, larvae were transferred from exposure dishes to well plates (one larvae/plate) and exposed to either substrate alone or substrate plus phorbol 12-myristate 13-acetate (PMA). In healthy fish PMA evokes respiratory burst response.	No effect on the innate immune system (measured as the respiratory burst response) of embryo-larval zebrafish. Response was measured for 2 hours in fish exposed to the 4 lowest doses and for 3.5 hours in fish exposed to the 2 highest doses	Elskus 2007 USGS and Maine Department of Environmental Protection

Appendix 6: Toxicity to aquatic invertebrates

Table A6-1: Acute Toxicity in Aquatic Invertebrates

Species	Exposure	Response	Reference
Daphnids			
Water flea, *Daphnia magna*, 24-hour-old instars	Spinosad with **50:50 mixture** of spinosad A: Spinosad D for 48 hours Mean measured concentrations: 0, 0.27, 0.53, 1.09, 2.29, 4.56, 9.53, 19.4, or 38.4 mg/L	48-hour LC_{50} >38.4 mg/L 48-hour EC_{50} = 7.37 mg/L	Cleveland et al. 2002b
Water flea, *Daphnia magna*, NOS	Spinosad (NOS) for 48 hours	48-hour EC_{50} >1.0 mg as/L (nominal)	European Commission 2006
Water flea, *Daphnia magna*, NOS	NAF-85 (NOS) for 48 hours	48-hour EC_{50} = 9.1 mg as/L	European Commission 2006
Water flea, *Daphnia magna*, <24 hours at initiation, 20/test concentration, 2 replicates/treatment level	Spinosad, technical grade (88% a.i.) under static conditions for 96 hours Nominal concentrations: 0, 0.0805, 0.115, 0.164, 0.234, 0.334, 0.477, 0.681, 0.973, 1.39, 1.99, 2.84, 4.05, 5. 78, 8.26, 11.8, 16.8, 24, 34.3, 49, 70, or 100 mg/L Mean measured concentrations: 0.021, 0.0269, 0.0411, 0.0585, 0.0846, 0.1333, 0.196, 0.303, 0.451, 0.633, 0.883, 1.28, 1.84, 2.7, 3.91, 5.69, 8.09, 11.8, 16.6, 23.71, 33.5, 48.2, 68.5 or 96.4 mg/L	96-hour EC_{50} =14.0 ppm NOAEC (mortality): ≈0.3 mg/L (see note below). Slightly toxic Working Note: The DER reports an investigator derived EC_{50} of 82.67 mg/L and an NOAEC 31.22 mg/L. The EPA reanalyzed the data and derived an EC_{50} of 14 mg a.i./L. Based on a 1-tailed Fisher Exact test, the NOAEC for mortality is 0.303 mg a.i./L based on a response of 4/20 at 0.451 mg a.i./L.	Milazzo et al. 1994 (DER) U.S.EPA/OPP/EFED 2009a, 2011a MRID 43414537/ 43574502 Acceptable Also summarized in Cleveland et al. 2002b
Formulations			
Water flea, *Daphnia magna*, neonates (<24-hours-old) at least in the F_3 generation, 20/concentration	Spinosad (Success®), 240 g a.i./L for 48 hours under static non-renewal conditions. Nominal concentrations: 0, 0.0001, 0.001, 0.01, 0.02, 0.1, 0.2, 0.4 mg a.i./L	48-hour LC_{50} = 0.0048 mg/L Nominal, not measured, concentration used to calculate the estimated LC_{50} value. See Table 2 of paper for slopes and confidence interval	Deardorff and Stark 2009 Washington State University, Sponsored by NOAA. This study is not summarized in ECOTOX.

Appendix 6: Toxicity to Aquatic Invertebrates *(continued)*

Species	Exposure	Response	Reference
Water flea, *Daphnia pulex*, neonates (<24-hours-old) at least in the F_3 generation, 20/concentration	Spinosad (Success®), 240 g a.i./L for 48 hours under static non-renewal conditions. Nominal concentrations 0, 0.1, 0.15, 0.25, 0.35, 0.5, 0.7 mg a.i./L `Working Note: Success is one of the representative formulations used in this risk assessment.`	48-hour LC_{50} = 0.129 mg/L Nominal, not measured, concentration used to calculate the estimated LC_{50} value. See Table 2 of paper for slopes and confidence interval	Deardorff and Stark 2009 Washington State University, Sponsored by NOAA This study is not summarized in ECOTOX.
Water flea, *Ceriodaphnia dubia*, neonates (<24-hours-old) at least in the F_3 generation, 20/concentration	Spinosad (Success®), 240 g a.i./L for 48 hours under static non-renewal conditions. Nominal concentrations: 0, 0.00005, 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1 mg a.i./L	48-hour LC_{50} = 0.0018 mg/L Nominal, not measured, concentration used to calculate the estimated LC_{50} value. See Table 2 of paper for slopes and confidence interval	Deardorff and Stark 2009 Washington State University, Sponsored by NOAA. This study is not summarized in ECOTOX.
Component Spinosyns			
Water flea, *Daphnia magna*	Spinosyn A (99%)	EC_{50}: >197 mg/L Practically nontoxic	U.S. EPA/OPP/EFED 2009a MRID 46505307 Acceptable
Water flea, *Daphnia magna*	Spinosyn D (100%)	EC_{50}: 66.8 mg/L Slightly toxic	U.S. EPA/OPP/EFED 2009a MRID 46505309 Acceptable
Water flea, *Daphnia magna*	Spinosyn D (96%)	EC_{50}: 3.8 mg/L Moderately toxic	U.S. EPA/OPP/EFED 2009a MRID 46505304 Acceptable
Metabolites			
Water flea, *Daphnia magna*	Spinosyn B (94%) [Demethylated Factor A]	48-hour EC_{50} = 6.5 ppm (mean measured) Moderately toxic	U.S. EPA/OPP/EFED 2009a, 2011a MRID 46505312 Acceptable Also summarized in European Commission 2006
Water flea, *Daphnia magna*	Spinosyn B (94%) [Demethylated Factor A]	48-hour EC_{50} = 6.39 ppm 48-hour LC_{50} = 21.4 ppm Moderately toxic	U.S. EPA/OPP/EFED 2009a MRID 44597731 Supplemental

Appendix 6: Toxicity to Aquatic Invertebrates *(continued)*

Species	Exposure	Response	Reference
Water flea, *Daphnia magna*	β-13,14-Dihydropseudoaglycone of factor D under static conditions for 48-hours	48-hour EC_{50} = 66.8 ppm Slightly toxic	U.S. EPA/OPP/EFED 2011a MRID 46505304 Acceptable Also summarized in European Commission 2006
Water flea, *Daphnia magna*	B-13-14-Dihydropseudoaglyconde of Spinosyn A for 48 hours	48-hour EC_{50} >197 ppm (mean measured) Practically nontoxic.	U.S. EPA/OPP/EFED 2011a MRID 46505307 Acceptable Also summarized in European Commission 2006
Water flea, *Daphnia magna*	N-Demethyl-D (the major degradate of spinosad factor D for 48 hours	48-hour EC_{50} = 3.7 ppm Moderately toxic European Commission (2006) indicates the toxicity value as 3.8 mg as/L (mean measured)	U.S. EPA/OPP/EFED 2011a MRID 46505309 Acceptable Also summarized in European Commission 2006
Water flea, *Daphnia magna*	Spinosad N-demethyl-A (the major degradate of spinosad A)	EC_{50} = 6.39 ppm Moderately toxic	US EPA/OPP/EFED 2009a, 2011a MRID 44597731 Supplemental
Mosquitos			
Mosquitoes, *Aedes aegypti*, larvae, late 3rd instars	Commercial formulation, (Tracer® Naturalyte Insect Control), 480 g/L spinosad Concentrations: 0, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3 or 1.0 mg a.i./L for 1 hour followed by 24-hour observation period.	1-hour LC_{50} = 0.06 mg a.i./L (estimated) Mortality scored based on non-responsiveness to gentle touching with a wood toothpick 24 hours post exposure	Antonio et al. 2008
Mosquitoes, *Aedes aegypti*, larvae, late 3rd instars raised to adults post exposure	Commercial formulation, (Tracer® Naturalyte Insect Control), 480 g/L spinosad Spinosad concentration: 0.06 mg a.i./L (estimated LC_{50} concentration determined in bioassay described above) for 1 hour. Surviving larvae were reared to adulthood.	The sublethal effects in surviving adult females included significantly larger wing length, greater production of eggs, and slightly less fertility, relative to control females. Surviving males were slightly smaller than controls. Treatment had no apparent effect on adult longevity.	Antonio et al. 2008

Species	Exposure	Response	Reference
Mosquitoes, *Culex pipiens* L., late 3rd and early 4th instars, 25/ concentration	Spinosad (Conserve® SC (Spinosyn A and Spinosyn D 120 g/L a.i.; NAF)	24-hour LC_{50} = 0.027 ppm (0.002-0.057 ppm) 24-hour LC_{90} = 0.111 ppm (0.054-5.383 ppm) Adult emergence was eliminated at concentrations >0.06 ppm	Cetin et al. 2005
Mosquitoes, *Aedes aegypti*, larvae, late 3rd instars, 5 lots per concentration	Spinosad, TGAI for 24 hours	**Strain** / **LC_{50} (mg/L)** SS: 0.35 RR: 0.32 See Table 1 of paper for confidence intervals and LC_{95} values.	Darriet et al. 2005
Mosquitoes, *Anopheles gambiae*, larvae, late 3rd instars, 5 lots per concentration	Spinosad, TGAI for 24 hours	**Strain** / **LC_{50} (mg/L)** SS: 0.01 RR: 0.011 See Table 1 of paper for confidence intervals and LC_{95} values.	Darriet et al. 2005
Mosquitoes, *Culex quinquefasciatus*, larvae, late 3rd instars, 5 lots per concentration	Spinosad, TGAI for 24 hours	**Strain** / **LC_{50} (mg/L)** SS: 0.093 RR: 0.12 See Table 1 of paper for confidence intervals and LC_{95} values.	Darriet et al. 2005
Mosquitoes, *Culex quinquefasciatus*, larvae, susceptible 2nd and 4th instars	Spinosad, technical powder (90.4% a.i.) designated as *old batch (lot QG28160W10)*	**Instar** / **24-hour LC_{50} (mg a.i./L)** / **48-hour LC_{50} (mg a.i./L)** 2nd: 0.021 / 0.019 4th: 0.033 / 0.026 See Table 1 of study for confidence intervals and LC_{90} values	Jiang and Mulla 2009
Mosquitoes, *Culex quinquefasciatus*, larvae, susceptible 2nd and 4th instars	Spinosad, technical powder (90.4% a.i.) designated as *new batch (lot RL02160W02)*	**Instar** / **24-hour LC_{50} (mg a.i./L)** / **48-hour LC_{50} (mg a.i./L)** 2nd: 0.024 / 0.019 4th: 0.031 / 0.027 See Table 1 of study for confidence intervals and LC_{90} values	Jiang and Mulla 2009
Mosquitoes, *Culex quinquefasciatus*, larvae, susceptible 2nd and 4th instars	Spinosad larvicidal liquid 120 SC (11.6% a.i.)	**Instar** / **24-hour LC_{50} (mg a.i./L)** / **48-hour LC_{50} (mg a.i./L)** 2nd: 0.012 / 0.010 4th: 0.014 / 0.013 See Table 1 of study for confidence intervals and LC_{90} values	Jiang and Mulla 2009

Appendix 6: Toxicity to Aquatic Invertebrates *(continued)*

Species	Exposure	Response	Reference
Mosquitoes, *Culex quinquefasciatus*, susceptible strain (Sebring-S) and field collected, larvae, 3rd instars, 20	Natular® 2EC 72 hour assay Sample label for this formulation specifies the a.i. as 20.6% mixture of spinosyn A and spinosyn D	Field collected: LC_{50} = 0.031 ppm Sebring-S strain (reference): LC_{50} = 0.028 ppm Resistance factor: ≈1.1	Jones and Ottea 2013
Mosquitoes, *Aedes albopictus*, larvae, 4th instar, 10 per concentration, 320 larvae tested in bioassay	Tracer® 24SC for 48 hours. For resistance studies, F1 or F2 generations from wild caught populations were used. No additional selection preassure.	48- hour LC_{50} = 0.019 µg/mL Resistance ratios were in the range of 23- to 50-fold, compared with the laboratory susceptible strain. Resistance to spinosad correlated with resistance to thiodicarb and indoxacarb but no correlation with several other pesticides including chlorpyurifos, cypermethrin, deltamethrin, lambdacyhalothrin, and emamectin benzoate.	Khan et al. 2011a Pakistan
Mosquitoes, *Aedes aegypti*, pupae and larvae (1st to 4th instars), 25/test	Spinosad, Obtained from T-Stanes & Company Limited, Research and Development Centre, Coimbatore, Tamil Nadu, India. Not clear if TGAI or formulation. Concentrations: 20, 40, 60, 80, or 100 ppm for 24 hours Study concerned with the larvicidal and pupicidal properties of spinosad against chikungunya vector	**Life stage** / **LC_{50} (ppm)** 1st Instar: 51.76882 2nd Instar: 61.87610 3rd Instar: 74.07166 4th Instar: 82.18527 Pupa: 93.44808 See Table 2 for percent mortality, LC_{90} values and confidence intervals. Working Note: The units of mg/L are correct.	Kovendan et al. 2012
Mosquitoes, *Anopheles stephensi*, larvae/pupae laboratory colony	Spinosad, from Kalpatharu pesticide Limited, India. Concentrations: 0.01. 0.02, 0.04, 0.06, or 0.08 ppm 24 hour exposure	**Life stage** / **24 h-LC_{50} (ppm)** 1st Instar: 0.002 2nd Instar: 0.003 3rd Instar: 0.028 4th Instar: 0.049 Pupa: 0.030 See Table 1 for percent mortality, LC_{90} values and confidence intervals	Kumar et al. 2011

Species	Exposure	Response	Reference
Mosquitoes, *Culex quinquefasciatus*, HAmCq, MAmCq, and VBFmCq strains, larvae, 4th instars, n=20	Spinosad (88% a.i.) for 24 hours. Wild caught strains versus laboratory (S-Lab) strain. No additional resistance pressure.	**Strain** / **24-hour LC_{50} (ppm)** / **Resistant Ratio** VBFmCq: 0.3: 3 HAmCq: 0.07: 0.7 MAmCq: 0.3: 3 S-Lab: 0.1: 1 See Table 1 of study for confidence intervals and LC_{90} values	Liu et al. 2004a Alabama
Mosquitoes, *Aedes albopictus*, HAmAal, MAmAal, VBFmAal, and SFmAal strains, larvae, 4th instars, n=20	Spinosad (88% a.i.) for 24 hours Wild caught strains versus laboratory (Ikaken) strain. No additional resistance pressure.	**Strain** / **24-hour LC_{50} (ppm)** / **Resistant Ratio** MAmAal: 0.2: 0.8 HAmAal: 0.2: 0.7 VBFmAal: 0.4: 1.3 SFmAal: 0.3: 1 Ikaken*: 0.3: *Susceptible laboratory strain See Table 1 of study for confidence intervals and LC_{90} values	Liu et al. 2004b Alabama
Mosquitoes, *Culex pipiens*, larvae (4th instar)	Spinosad (Tracer® 12% SC) for 24 hours Test solutions prepared on the basis of a.i. content	24-hour LC50: 0.087 mg/L See Table 1 for LC_{25} values and fiducial limits	Mansour et al. 2012
Mosquitoes, *Aedes aegypti*, 25 late 3rd and early 4th instars, 4 larvae/treatment group	Tracer Naturalyte® Insect Control containing 480 g/L a.i. for 1 hour Concentrations: 0.001, 0.003, 0.01, 0.03, or 0.1 mg a.i./L	1-hour LC_{50} = 0.026 ppm (estimated)	Perez et al. 2007
Mosquitoes, *Aedes aegypti*, *Anopheles stephensi*, and *Culex pipien*, 3rd instars, 20/concentration, at least 3 replicates	Laser® (4.8% emulsifiable concentrate. Concentration: 0.001 to 0.1 mg/L for 24 and 48 hours	**Species** / **24-hour LC_{50} (mg/L)** / **48-hour LC_{50} (mg/L)** *An. stephensi*: 0.039: 0.024 *Ae. aegypti*: 0.0096: 0.0070 *Cx. pipiens*: 0.0064: 0.0032 See Table 1 of study for LC_{90} and LC_{99} values	Romi et al. 2006

Appendix 6: Toxicity to Aquatic Invertebrates *(continued)*

Species	Exposure	Response	Reference
Mosquitoes, *Culex quinquefasciatus*, larve, late 3rd instars, n=25, 45 generations	Natular XRG (2.5% a.i.), sand granules for 24 hours Study entailed successive selections for 45 generations (i.e., Selected Colony). Susceptibility changes in response to selection were determined every other generation. Selection pressure: LC_{70-90} over 45 generations. Test period: 24 hours	**With Selection Pressure:** F_1: LC_{50}: 0.671 mg/L F_{45}: LC_{50}: 693.5 mg/L Resistance factor: 1033 **Wild Population (no selection pressure:** F_1: LC_{50}: 0.250 mg/L F_{45}: LC_{50}: 0.490 mg/L Range of LC_{50}s: 0.196 to 0.490 mg/L. (factor of 2.5) **Reference Lab Culture (no selection pressure):** F_1: LC_{50}: 0.272 mg/L F_{45}: LC_{50}: 0.311 mg/L Range of LC_{50}s: 0.234 to 0.424 mg/L. (factor of 1.8)	Su and Chen 2014b
Mosquitoes, *Culex quinquefasciatus*, larvae, 3rd instars, 25/ concentration	Natular T30 (8.33% a.i.) Concentrations: 0.0045, 0.0030, 0.0060, or 0.0250 ppm for 24 hours	24-hour LC_{50} =0.0067 ppm	Su et al. 2014
Other Standard Lethality Studies			
Non-biting midge, *Chironomus circumdatus*, larvae/pupae laboratory colony	Spinosad, from Kalpatharu pesticide Limited, India. Formulation or a.i. not specified. Concentrations: 0.01. 0.02, 0.04, 0.06, or 0.08 ppm	**Life stage** / **24-hour LC_{50} (ppm)** 1st Instar: 0.009 2nd Instar: 0.015 3rd Instar: 0.032 4th Instar: 0.053 Pupa: 0.049 See Table 1 for percent mortality, LC_{90} values and confidence intervals	Kumar et al. 2011
Eastern oyster, *Crassostrea virginica*,	Spinosad, TGAI, for 96 hours under continuous flow conditions Mean measured concentrations: 0, 0.093, 0.114, 0.222, 0.333, or 0.527 mg/L	96-hour EC_{50} = 0.3 ppm (based on new shell growth)	US EPA/OPP/EFED 2011a MRID 43444104/ 43571203 Acceptable Cleveland et al. 2002b
Amphipod, *Leptocheirus plumulosus*	Spinosad, TGAI, whole sediment toxicity study for 10 days	**Pore water** NOAEC = 1.38 mg total residue/L **Sediment** NOAEC = 115 mg total residue/L	U.S. EPA/OPP/EFED 2011a MRID 47702901 Supplemental

Appendix 6: Toxicity to Aquatic Invertebrates *(continued)*

Species	Exposure	Response	Reference
Grass shrimp, *Palaemonetes pugio*, NOS	Spinosad, 87.9% a.i., 96 hours under static renewal conditions Mean measured concentrations: 0, 1.66, 2.71, 4.00, 6.19, or 9.76 mg/L	96-hour LC_{50} >9.76 ppm	U.S. EPA/OPP/EFED 2005, MRID 434145-39 Supplemental Also cited in Cleveland et al. 2002b
Non-Standard Studies			
Aquatic insects: Ephemeroptera (4 families, 5 species), Odonata (4 families, 4 species), Trichoptera (3 families, 3 species) and Hemiptera (2 families, 2 species). See Table 1 of paper	SpinTor 12SC, 12 ppm (12 mg a.i./L) for 10 minutes followed by incubation with 10 mL aerated river water for 5 hours.	Mortality was not significantly increased over untreated controls	Infante-Rodriguez et al. 2011
Aquatic insects: Plecoptera	SpinTor 12SC, 12 ppm (12 mg a.i./L) for 10 minutes followed by incubation with 10 mL aerated river water for 5 hours.	Significant increase in mortality ($P<0.001$) over untreated controls, but considered moderate (59% versus 19% in controls) by the investigators.	Infante-Rodriguez et al. 2011
Blackflies, *Simulium* spp., late-instar larvae 20/concentration	SpinTor 12SC Nominal concentrations: (untreated river water control), 0.2, 0.4, 0.8, 1.6, or 3.2 mg a.i./L for 10 minute exposure followed by incubation with 10 mL aerated river water for 5 hours.	15-74% mortality observed at 5 hours post exposure 10-minute LC_{50} = 1.48 mg a.i./L.	Infante-Rodriguez et al. 2011
Damselflies *(Ischnura* sp., n=38); Dragonflies, *(Pachydiplax* l*ongipennis*, n=28); and Mayflies (*Caenis* sp., n=29) *Representative nontarget organisms based on abundance at collection site.*	Natular® 2EC 0.031 ppm (LC_{50} value determined by investigators for field collected mosquitoes) or 1.6 ppm (equivalent to the maximum label rate (2.8 fl oz./acre) for Natular 2EC. Sample label for this formulation indicates that the a.i. is 20.6% as a mixture of spinosyn A and spinosyn D	There was a marked difference in susceptibility among nontarget taxa. Susceptibility was greatest in mayflies., followed by damselfies., and then dragonfies See Figure 2 of study for mean mortality values indicated by bars representing concentrations.	Jones and Ottea 2013

Table A6-2: Chronic toxicity in Aquatic Invertebrates

Species	Exposure	Response	Reference
Daphnids			
Spinosad			
Water flea, *Daphnia magna*, Instars, <24-hours-old, 5/replicate, 4 replicates	Spinosad (88% a.i.) under flow-through conditions for 21 days Nominal concentrations: 0, (solvent control), 0.6, 1.1, 1.7, 2.8, 4.6, or 7.7 µg/L Mean measured concentrations: 0.392, 0.617, 1.15, 2.19, 3.96, or 5.84 µg/L	21-day LC_{50} >56.6 µg/L NOAEC = 0.62 µg/L LOAEC = 1.2 µg/L LOAEC for specific effects: Egg production = 2.19 ppb Growth (length) = 1.15 ppb	U.S. EPA/OPP/ EFED 2011a MRID 43848801 Acceptable Cleveland et al. 2002b
Water flea, *Daphnia magna*, NOS	Spinosad, NOS, under flow-through conditions (5-day pulsed) Mean measured concentrations: 0.919, 1.77, 3.69, 6.88, 14.4, 28.6, or 56.6 µg/L	21-day LC_{50} >56.6 µg/L NOEC = 6.88 µg/L Working Note: Not a standard reproduction study. Discuss in text but do not put in summary table of reproduction studies.	Cleveland et al. 2002b
Water flea, *Daphnia magna*, NOS	Spinosad (NOS) for 21 days under flow-through or semi-static conditions	Flow-through NOEC = 0.0012 mg/L [1.2 µg a.i./L] (mean measured) Static renewal NOEC = 0.0080 mg/L [8 µg a.i./L] (nominal)	European Commission 2006
Water flea, *Daphnia magna*, <24 hours old, 5 replicates	Conserve® 120SC (11.6% a.i.) Concentration: 8 µg/L Duration: 14 days Static renewal, every 2 days.	Decreased survival (Figure 1a of study). Significant decrease in fecundity from Days 8 to 10.	Duchet et al. 2010b
Water flea, *Daphnia pulex*, <24 hours old, 5 replicates	Conserve® 120SC (11.6% a.i.) Concentration: 8 µg/L Duration: 14 days Static renewal, every 2 days.	Decreased survival (Figure 1b of study). Significant decrease in fecundity only on Day 8.	Duchet et al. 2010b
Water flea, *Daphnia magna*, 4-6th instars (<24-hours-old)	Spinosad, Conserve® 120 SC (11.6% a.i.) for 14 days Nominal concentrations: 2, 4, or 8 µg/L Average exposure concentrations: 0.23, 0.50, or 0.62 µg/L	2 µg/L: Transient decrease in number of offspring (Days 8 to 12). No effect on number of adults. 4 µg/L: Decrease in number of offspring only on Day 8. Decrease in number of adults over all durations. 8 µg/L: Decrease in number of adults and offspring from Day 8 to Day 14. See Figure 3 of paper.	Duchet et al. 2011

Appendix 6: Toxicity to Aquatic Invertebrates *(continued)*

Species	Exposure	Response	Reference
Water flea, *Daphnia pulex*, neonates, 30/concentration	Spinosad (Success®), 240 g a.i./L for 8 days Concentrations: 0, 2, 4, 6, 8, 10, and 11 µg a.i./L. Static renewal (every other day). Survival and reproduction measured every 24 hours until all animals died. At lower concentrations, observations lasted up to about 70 days (Figure 1 of paper).	Concentration related decrease in populations (several metameters) at all concentrations (Figures 1 to 4 of paper).	Stark and Vargas 2003
Water flea, *Daphnia pulex*, 4-6th brood offspring (<24-hours-old)	Spinosad, Conserve® 120 SC (11.6% a.i.) for 14 days Nominal concentrations: 2, 4, or 8 µg/L Average exposure concentrations: 0.23, 0.50, or 0.62 µg/L	Decreases in numbers of adults and offspring from Day 8 to Day 12 at all concentrations.	Duchet et al. 2011
Water fleas, *Daphnia pulex*, n=300	Spinosad (NOS) Exposure to 129 µg/L for 10 days	No organisms survived. No reproduction.	Stark 2005
Water flea, *Ceriodaphnia dubia*, ≤24-hours-old, 20/concentration	Spinosad (Success®), 240 g a.i./L for 8 days Nominal concentrations: 0, 0.5, 1, 2.5, or 10 µg a.i./L Authors indicate that spinosad appears to adversely affect *C.* dubia at or near the expected environmental concentration of 2.3µg/L. Working Note: Estimated upper bound concentrations in current risk assessment are higher than 2.3 µg/L	0.5 µg/L: NOAEC. ≥1.0 µg/L Significantly reduced the final number of individuals, and population growth rate. ≥2.5 µg/L: Significant increase in mortality of founders (individuals used to start exposure study) and the number of offspring and surviving females at concentrations. 10 µg/L: Population decline	Deardorff and Stark 2011 Washington State University, Sponsored by NOAA This study is not summarized in ECOTOX.

Appendix 6: Toxicity to Aquatic Invertebrates *(continued)*

Species	Exposure	Response	Reference
Metabolites			
Water flea, *Daphnia magna*, NOS	β-13,14-Dihydropseudo-aglycone of spinosad factor D for 21 days under flow-through and semi-static conditions	NOAEC (length) = 4.85 ppm	US EPA/OPP/EFED 2011a MRID 46505303 Supplemental Also summarized in European Commission 2006
Water flea, *Daphnia magna*, NOS	β-13,14-Dihydropseudo-aglycone of spinosad factor A for 21 days under flow-through and semi-static conditions	NOAEC (length) = 1.59 ppm	US EPA/OPP/EFED 2011a MRID 46505306 Supplemental Also summarized in European Commission 2006
Water flea, *Daphnia magna*, NOS	Spinosyn B for 21 days under flow-through conditions	NOEC = 0.00095 mg a.i./L [0.95 µg a.i./L] (mean measured)	European Commission 2006
Water flea, *Daphnia magna*, NOS	N-demethylated spinosyn D for 21 days under flow-through and semi-static conditions	NOEC = 0.001 mg a.i./L [1 µg a.i./L] (mean measured)	European Commission 2006
Midges			
Spinosad			
Freshwater midge, *Chironomus riparius*, larvae	Spinosad, Factor A & D, in sediment for 25 days	**Sediment** NOAEC = 1.14 ppb **Overlying water** NOAEC = 0.622 ppb Reduced adult emergence at 1.328 ppb	US EPA/OPP/EFED 2011a MRID 44828402 Supplemental
Freshwater midge, *Chironomus riparius*, NOS	Spinosad, NOS, for 25 days under static conditions Nominal concentrations: 0, 0.1, 0.2, 0.4, 0.8, 1.6, or 3.2 µg/L	21-day EC_{50} > 3.2 µg/L NOEC = 1.6 µg/L LOEC = 3.2 µg/L	Cleveland et al. 2002b
Freshwater midge, *Chironomus riparius*, larvae	Spinosad (NOS) for 25 days	NOEC = 0.0016 mg/L (initial measured concentration in overlying water)	European Commission 2006 Possibly the same data as Cleveland et al 2002b in above entry

Appendix 6: Toxicity to Aquatic Invertebrates *(continued)*

Species	Exposure	Response	Reference
Components			
Freshwater midge, *Chironomus riparius*, larvae, male and female	Spinosad A Test conducted under static conditions in overlying water spiked exposure (sediment not spiked) for 28 days	No effect on development rate and emergence. NOAEC = 0.0734 ppm degradate LOAEC >0.0734 ppm degradate EC_{50} >0.0734 ppm degradate *Toxicity values based on mean-measured pore water treatment concentrations*	US EPA/OPP/EFED 2011a MRID 46505314 Non-guideline
Metabolites			
Freshwater midge, *Chironomus riparius*, larvae	Spinosyn B for 28 days	NOEC = 0.0032 mg/L (initial measured concentration in overlying water)	European Commission 2006
Freshwater midge, *Chironomus riparius*, larvae, male and female	N-demethyl-D Test conducted under static conditions in overlying water spiked exposure (sediment not spiked) for 28 days	No significant reductions in development rates (male, female and combined sexes), relative to controls; no additional sublethal effects for controls or treatment groups. NOAEC = 0.14 ppb a.i. `Working Note: The indefinite NOAEC is somewhat lower than the NOAEC of 0.622 µg a.i./L for spinosad.` LOAEC: not defined EC_{50} >0.14 ppb a.i. *Toxicity values based on mean-measured pore water treatment concentrations*	US EPA/OPP/EFED 2011a MRID 46505315 Non-guideline
Freshwater midge, *Chironomus riparius*, NOS	N-demethylated spinosyn D for 28 days	NOEC = 0.0024 mg as/L (mean measured initial concentration in overlying water)	European Commission 2006
Freshwater midge, *Chironomus riparius*, NOS	β-13,14-Dihydropseudoaglycone of Spinosyn A for 28 days	NOEC ≥1.120 mg as/L (mean measured initial concentration in overlying water)	European Commission 2006

Appendix 6: Toxicity to Aquatic Invertebrates *(continued)*

Species	Exposure	Response	Reference
Freshwater midge, *Chironomus riparius*, larvae, male and female	β-13,14-Dihydropseudoaglycone of Spinosad D Test conducted under static conditions in overlying water spiked exposure (sediment not spiked) for 28 days	No statistically significant ($p<0.05$) or biologically significant reductions in male or female development rates and % emerged, relative to controls; no additional sublethal effects (abnormal behavior) for controls or treatment groups. NOAEC = 0.0388 ppm degradate LOAEC >0.0388 ppm degradate EC_{50} >0.0388 ppm degradate *Toxicity values based on mean-measured pore water treatment concentrations*	US EPA/OPP/EFED 2011a MRID 46505316 Non-guideline
Freshwater midge, *Chironomus riparius*, NOS	β-13,14-Dihydropseudoaglycone of Spinosyn D for 28 days	NOEC ≥0.731 mg as/L (mean measured initial concentration in overlying water)	European Commission 2006
Freshwater midge, *Chironomus riparius*, larvae, male and female	N-demethyl-A Test conducted under static conditions in overlying water spiked exposure (sediment not spiked) for 28 days	No significant reductions in development rates (male, female and combined sexes), relative to controls; no additional sublethal effects (abnormal behavior) for controls or treatment groups. NOAEC = 0.41 ppb a.i. `Working Note: The NOAEC is somewhat lower than the NOAEC of 0.622 µg a.i./L for spinosad.` LOAEC >0.41 ppb a.i. EC_{50} >0.41 ppb a.i. *Toxicity values based on mean-measured pore water treatment concentrations*	US EPA/OPP/EFED 2011a MRID 46505317 Non-guideline
Mosquitoes			
Mosquitoes, *Aedes aegypti*, 3rd instar larvae, 25/concentration	Tracer EC (480 g a.i./L) concentration suspension for 10 days Concentrations: 0.0, 0.025, 0.05, 0.1, 0.5, 1.0, 4.0 or 10.0 ppm	Concentrations as low as 0.5 ppm led to 100% mortality in less than 5 days.	Tome et al. 2014

Appendix 6: Toxicity to Aquatic Invertebrates *(continued)*

Species	Exposure	Response	Reference
Mosquitoes, *Aedes aegypti*, 4th instar larvae (24 hours after exposure of 3rd instar larvae to spinosad) and 1-day-old pupae (96 hours after exposure of the 3rd instar larvae), 20 larvae and 20 pupae/concentration	Tracer EC (480 g a.i./L) concentration suspension for 10 days Concentrations: 0.0, 0.025, 0.05, 0.1, 0.5, 1.0, 4.0 or 10.0 ppm	Sublethal exposure compromised juvenile swimming described as reduced swimming speed and wriggling movements in both 4th instar larvae and pupae.	Tome et al. 2014
Mosquitoes, *Aedes aegypti*, five 4th instar larvae (24 hours after exposure of 3rd instar larvae to spinosad) and five 1-day-old pupae (96 hours after exposure of the 3rd instar larvae)	Tracer EC (480 g a.i./L) concentration suspension for 10 days Concentrations: 0.0, 0.025, 0.05, 0.1, 0.5, 1.0, 4.0 or 10.0 ppm	No evidence of DNA fragmentation in neuromuscular cells of 4th instar larvae or pupae.	Tome et al. 2014
Shrimp			
Mysid shrimp, *Mysidopsis bahia*,	Spinosad, TGAI for 28 days under flow-through conditions Mean measured concentrations: 84.2, 173, 360, 713, or 1470 ppm	Reduction in number of young/female: NOAEC = 0.0842 ppm LOAEC = 0.173 ppm MATC = 0.120 ppm	US EPA/OPP/EFED 2011a MRID 44420602 Acceptable Cleveland et al. 2002b

Table A6-3: Microcosm/Mesocosm Studies in Aquatic Invertebrates

Species	Exposure	Response	Reference
Daphnids			
Water flea, *Daphnia pulex*, isolated natural populations. Microcosms (125 L bottomless enclosures, stabilized for 24 hours prior to treatments), 5 replicates per concentration, placed in a shallow temporary brackish marsh in Western France. Microcosms contained algae (NOS), and mixed species of crustaceans (Table 1 of paper).	Spinosad 120 SC, 120 g a.i./L. Observations on Days 0, 2, 4, 7, 14 and 21 days after treatment. <u>Nominal concentrations for 30 cm water depth</u>: 8, 17, or 33 µg/L (5 replicates per concentration). Concentrations intended to reflect applications of 25, 50, or 100 g/ha. Concentrations based on nominal water depth of 30 cm	Sharp decrease in daphnid abundance at all concentrations. Recovery to near Day 0 levels at the lowest concentration by Day 14 (Figure 3 of paper). Concentration related decrease in body length starting on Day 2 of study (Table 2 of paper). Working Note: Study authors note that the decreases in populations are not consistent with standard acute EC_{50}s. Suggest that greater sensitivity in microcosms may be due to decrease in oxygen levels in water. The LOAEL of 17 µg/L, however, is consistent with the chronic toxicity data on *D. pulex – i.e., LOAELs of 2 and 8 µg/L in Table 26 of current risk assessment*.	Duchet et al. 2008
Water flea, *Daphnia magna*, isolated natural populations. Microcosms (125 L bottomless enclosures, stabilized for 24 hours prior to treatments), 5 replicates per concentration, placed in a shallow temporary brackish marsh in Western France.	Spinosad 120 SC, 120 g a.i./L. Observations on Days 0, 2, 4, 7, 14 and 21 days after treatment. <u>Nominal concentrations for 30 cm water depth</u>: 8, 17, or 33 µg/L (5 replicates per concentration). Concentrations intended to reflect applications of 25, 50, or 100 g/ha. Concentrations based on nominal water depth of 30 cm.	Sharp decrease in daphnid abundance at all concentrations. No recovery. Concentration related decrease in body length starting on Day 2 of study (Table 2 of paper). Variations in water temperature and salinity had a significant effect on the abundance of *D. magna*. The authors suggest that *the peak of salinity observed during the 21-day observation period may have been partly responsible for the absence of recovery in the microcosms*. Working Note: This study is consistent with LOAELs in *D. magna* from chronic studies. See Table 26 of current risk assessment.	Duchet et al. 2010a

Appendix 6: Toxicity to Aquatic Invertebrates *(continued)*

Species	Exposure	Response	Reference
Other Invertebrates			
Non-biting midges, *Polypedilum nubifer* and *Tanytarsus curticornis* [Diptera: Chironomidae]. Microcosms (125 L bottomless enclosures, stabilized for 24 hours prior to treatments), 5 replicates per concentration, placed in a shallow temporary brackish marsh in Western France.	Spinosad 120 SC, 120 g a.i./L. Observations on Days 0, 2, 4, 7, 14 and 21 days after treatment. Nominal concentrations for 30 cm water depth: 8, 17, or 33 µg/L (5 replicates per concentration). Concentrations intended to reflect applications of 25, 50, or 100 g/ha. Concentrations based on nominal water depth of 30 cm.	*Polypedilum nubifer*: Significant decrease in emergence starting on Day 4 at all concentrations (Table 1 of paper). *Tanytarsus curticornis* Decrease in adult emergence at 17 and 33 µg/L but not statistically significant, relative to controls. No effect at 8 µg/L. See Figure 2 of paper. Working Note: The LOAEL of 8 µg/L is consistent with all of the chronic studies in *C. riparius*. See Table 26 of current risk assessment.	Duchet et al. 2015
Mosquitoes, *Culex tarsalis*, larvae (2nd instar) [Diptera: Culicidae] (target species) and chironomid midge larvae [Diptera: Chironomidae] 1,150 liter mesocosms, mud substrate with added vegetation. 10-30 cm in depth	Natular® formulations of spinosad: G30 granules (sustained release) and 2EC liquid in 15 wetland mesocosms constructed in cattle watering tanks in Yolo County, CA. Five mesocosms/treatment 42 day observation period. Natular® G30 granules were applied by gloved hand at 14.57 kg/ha (13 lb/acre) – midrange of label rate Natular® 2EC liquid was applied by hand sprayer at 204.58 mL/ha (2.8 oz/acre) – maximum label rate	Both formulations of spinosad were highly effective against *Culex tarsalis*, larvae, and strongly suppressed chironomid midge larvae. Mortality rates of 52.4 to >96 % (Table 1 of paper) Working Note: Paper does not provide estimates of the concentration of spinosad in water. This study, however, can be used to assess sensitivity of Diptera relative to Ephemeroptera. See entry below for Ephemeroptera.	Lawler and Dritz 2013

Appendix 6: Toxicity to Aquatic Invertebrates *(continued)*

Species	Exposure	Response	Reference
Mayflies, *Callibaetis californicus* [Ephemeroptera: Baetidae] nymphs, 3-5 cm long, 8-10/replicate 1,150 liter mesocosms, mud substrate with added vegetation. 10-30 cm in depth t	Natular® formulations of spinosad: G30 granules (sustained release) and 2EC liquid in 15 wetland mesocosms constructed in cattle watering tanks in Yolo County, CA. 42 day observation period. Natular® G30 granules were applied by gloved hand at 14.57 kg/ha (13 lb/acre) – midrange of label rate Natular® 2EC liquid was applied by hand sprayer at 204.58 mL/ha (2.8 oz/acre) – maximum label rate	Mayfly nymphs were less sensitive than the target species, *Culex tarsalis* and midge larvae. Mortality rates of 26 to 94% (Table 1 of paper). Working Note: Paper does not provide estimates of the concentration of spinosad in water. This study, however, can be used to assess sensitivity of Diptera relative to Ephemeroptera. See entry above for Diptera. Lesser sensitivity of Ephemeroptera relative to Diptera consistent with short-term study by Infante-Rodriguez et al. (2011).	Lawler and Dritz 2013
Mosquitoes, *Culex quinquefasciatus*, Diptera: Culicidae, natural populations of 3rd and 4th instars, but no pupae. Outdoor microcosms.	Spinosad 120 SC (11.6% a.i.) diluted with distilled water to 1.16% a.i.) in outdoor tubs. 35 day observation period. Concentrations: 0.05, 0.1, 0.25, or 0.5 mg a.i./L Equivalent applications: 0.1, 02, 0.5, or 1 lb a.i./acre Treatments made 7 days after flooding. Sampling by dipping technique before and 1, 4, 7, 14, 21, 28, and 35 days after treatment to assess initial and persistent efficacy.	Control of immature *Culex* spp. for 21 days at concentrations of 0.05 mg a.i./L and 35 days at 0.1 to 0.5 mg a.i./L. Working Note: This efficacy trial consistent with the acute LC_{50} values from this study – i.e., 48 hour LC_{50} values of about 0.01 mg a.i./L. for *C. guinquefasciatus*. See Table A6-1.	Jiang and Mulla 2009
Mosquitoes, *Culex quinquefasciatus*, Diptera: Culicidae, natural populations of early and late instars, and few pupae. Outdoor microcosms.	Spinosad 120 SC (11.6% a.i.) diluted with distilled water to 1.16% a.i.) applied to the water surface of 12 bare-ground dirt ponds 8 days after flooding. 14 day observation period. Application rates: 0.025, 0.05, and 0.1 mg a.i./L, equal to 0.067, 0.133, and 0.267 lb a.i./acre Sampling done by dipping technique before and 1, 4, 7, and 14 days after treatment to assess initial and persistent efficacy	Control of *Culex* mosquitoes for 14 days or longer at 0.025 to 0.1 mg a.i./liter. Working Note: This efficacy trial consistent with the acute LC_{50} values from this study – i.e., 48 hour LC_{50} values of about 0.01 mg a.i./L. for *C. guinquefasciatus*. See Table A6-1.	Jiang and Mulla 2009

Appendix 7: Toxicity to Aquatic Plants

Table A7-1: Algae

Species	Exposure	Response	Reference
Spinosad			
Freshwater diatom, *Navicula pelliculosa*	Spinosad (88% a.i.) for 120 hours	EC_{50} = 0.09 mg/L (cell density) NOAEC = 0.05 mg/L	U.S. EPA/OPP /EFED 2011a MRID 43414543 Acceptable
Freshwater diatom, *Navicula pelliculosa*	Spinosad, NOS, under static conditions for 5 days Mean measured concentrations: 0, 0.011, 0.031, 0.049, or 0.12 (0.340)	EC_{50} = 0.135 mg/L EC_{25} = 0.113 mg/L NOEC = 0.049 mg/L	Cleveland et al. 2002b
Freshwater diatom, *Navicula pelliculosa*	Spinosad (NOS) for 120 hours (5 days)	EC_{50} = 0.079 mg a.i./L (mean measured)	European Commission 2006
Freshwater diatom, *Navicula pelliculosa*	NAF-85 (Tracer® formulation, 44.2% a.i) for 120 hours (5 days)	EC_{50} = 0.35 mg a.i./L (mean measured)	European Commission 2006
Marine diatom, *Skeletonema costatum*	Spinosad (88% a.i.) under static conditions for 5 days Mean measured concentrations: 0, 0.167, 0.342, 0.774, 1.56, or 3.27 mg/L	EC_{50} = 0.227 mg/L EC_{25} = 0.143 mg/L NOEC = 0.167 mg/L	Cleveland et al. 2002b
Blue/green alga, *Anabaena flos-aquae*	Spinosad for 120 hours (5 days)	EC_{50} = 6.1 mg as/L (nominal)	European Commission 2006
Blue/green alga, *Anabaena flos-aquae*	Spinosad, (88% a.i.), under static conditions for 5 days Mean measured concentrations: 0, 1.8, 3.9, 7.9, 16.3, or 26.6 mg/L	EC_{50} = 8.09 mg/L EC_{25} = 6.33 mg/L NOEC = 3.89 mg/L	Cleveland et al. 2002b
Green alga, *Selenastrum capricornutum*	Spinosad (88.2% a.i.), under static conditions for 7 days Mean measured concentrations: 0, 4.3, 11.1, 12.2, 20.3, 35.6, 60.8, or 105.5 mg/L	EC_{50} >105.5 mg/L NOEC = 4.3 mg/L	Cleveland et al. 2002b
Green alga, *Selenastrum capricornutum*	NAF-85 (Tracer® formulation, 44.2% a.i) for 120 hours	EC_{50} > 48 mg a.i./L (nominal)	European Commission 2006

Appendix 7: Toxicity to Aquatic Plants *(continued)*

Species	Exposure	Response	Reference
Metabolites			
Freshwater diatom, *Navicula pelliculosa*	Spinosyn B (NOS) for 120 hours	EC_{50} = 0.077mg a.i./L (mean measured)	European Commission 2006 Working Note: This study is not included in EPA risk assessments.
Freshwater diatom, *Navicula pelliculosa*	Degradate of Factor A (β-13,14-dihydropseudo-aglycone)	EC_{50} = 31 ppm (biomass) NOAEC = 8.34 ppm (cell density)	U.S. EPA/OPP /EFED 2011a MRID 46505305 Supplemental
Freshwater diatom, *Navicula pelliculosa*	β-13,14-dihydropseudo-aglycone of spinosad D for 96 hours	EC_{50} = 28 mg as/L (mean measured)	European Commission 2006
Freshwater diatom, *Navicula pelliculosa*	β-13,14-dihydropseudo-aglycone of spinosyn A for 72 hours	EC_{50} = 38.8 mg as/L (nominal)	European Commission 2006
Freshwater diatom, *Navicula pelliculosa*	β-13,14-dihydropseudo-aglycone of spinosad D	EC_{50} = 19 ppm (growth inhibition) (*based on the area under the curve*) NOAEC = 14.2 ppm	U.S. EPA/OPP /EFED 2011a MRID 46505302 Supplemental
Freshwater diatom, *Navicula pelliculosa*	N-demethyl-D	EC_{50} = 0.22 ppm (cell density) NOAEC = 0.17 ppm	U.S. EPA/OPP /EFED 2011a MRID 46505308 Supplemental
Freshwater diatom, *Navicula pelliculosa*	N-demethylated spinosyn D for 120 hours	EC_{50} = 0.25 mg as/L (mean measured)	European Commission 2006
Freshwater diatom, *Navicula pelliculosa*	N-demethyl-A	EC_{50} = 0.16 ppm (cell density) NOAEC <0.019 ppm	U.S. EPA/OPP /EFED 2011a MRID 46505310 Supplemental

Table A7-2: Macrophytes

Species	Exposure	Response	Reference
Duckweed, *Lemna gibba*	Spinosad, (88% a.i.) for 7 days under static conditions <u>Mean measured concentrations</u>: 0, 0.52, 1.0, 1.9, 4.3, 7.4, or 14.3 mg/L	$EC_{50\ (growth)}$ = 10.6 ppm NOAEC = 1.86 ppm	U.S. EPA/OPP /EFED 2011a MRID 43414546 Acceptable Cleveland et al. 2002b

One Application

Table A8-1: Effective Offsite Application Rate (lb/acre)

Site	Clay	Loam	Sand
Dry and Warm Location	0.00134 (0 - 0.0102)	0 (0 - 0.00256)	0 (0 - 0)
Dry and Temperate Location	0.00165 (1.91E-05 - 0.0254)	0.000061 (0 - 0.0055)	0 (0 - 0.00076)
Dry and Cold Location	0.00041 (0.000036 - 0.005)	0 (0 - 0.000156)	0 (0 - 0)
Average Rainfall and Warm Location	0.046 (0.0171 - 0.153)	0.0182 (0.0037 - 0.065)	0.00139 (0 - 0.0185)
Average Rainfall and Temperate Location	0.029 (0.0108 - 0.155)	0.0101 (0.00191 - 0.048)	0.000244 (0 - 0.014)
Average Rainfall and Cool Location	0.0191 (0.009 - 0.099)	0.0039 (0.00115 - 0.0178)	0 (0 - 0.0032)
Wet and Warm Location	0.125 (0.061 - 0.307)	0.081 (0.037 - 0.197)	0.0126 (0.00303 - 0.067)
Wet and Temperate Location	0.11 (0.059 - 0.314)	0.056 (0.0281 - 0.139)	0.0055 (0.00069 - 0.032)
Wet and Cool Location	0.106 (0.043 - 0.299)	0.038 (0.016 - 0.111)	0.00238 (0.00033 - 0.0148)
Average of Central Values:	0.049	0.023	0.00246
25th Percentile:	0.00165	6.10E-05	0
Maximum:	0.314	0.197	0.067
Summary:	0.049 (0.00165 - 0.314)	0.023 (6.10E-05 - 0.197)	0.00246 (0 - 0.067)

Appendix 8: GLEAMS-Driver, Single Application

One Application

Table A8-2: Concentration in Top 12 Inches of Soil (ppm)

Site	Clay	Loam	Sand
Dry and Warm Location	0.37 (0.36 - 0.38)	0.34 (0.33 - 0.35)	0.34 (0.33 - 0.35)
Dry and Temperate Location	0.37 (0.36 - 0.38)	0.34 (0.33 - 0.35)	0.34 (0.33 - 0.35)
Dry and Cold Location	0.38 (0.36 - 0.38)	0.35 (0.33 - 0.35)	0.35 (0.33 - 0.35)
Average Rainfall and Warm Location	0.36 (0.34 - 0.37)	0.34 (0.32 - 0.34)	0.34 (0.33 - 0.34)
Average Rainfall and Temperate Location	0.37 (0.34 - 0.38)	0.34 (0.32 - 0.35)	0.34 (0.33 - 0.35)
Average Rainfall and Cool Location	0.37 (0.35 - 0.38)	0.35 (0.33 - 0.35)	0.35 (0.33 - 0.35)
Wet and Warm Location	0.35 (0.312 - 0.36)	0.33 (0.308 - 0.34)	0.34 (0.32 - 0.34)
Wet and Temperate Location	0.36 (0.33 - 0.37)	0.34 (0.32 - 0.34)	0.34 (0.33 - 0.35)
Wet and Cool Location	0.36 (0.33 - 0.37)	0.34 (0.33 - 0.35)	0.35 (0.33 - 0.35)
Average of Central Values:	0.37	0.34	0.34
25th Percentile:	0.36	0.34	0.34
Maximum:	0.38	0.35	0.35
Summary:	0.37 (0.36 - 0.38)	0.34 (0.34 - 0.35)	0.34 (0.34 - 0.35)

One Application

Table A8-3: Concentration in Top 36 Inches of Soil (ppm)

Site	Clay	Loam	Sand
Dry and Warm Location	0.124 (0.119 - 0.125)	0.114 (0.109 - 0.115)	0.114 (0.109 - 0.115)
Dry and Temperate Location	0.125 (0.12 - 0.126)	0.115 (0.11 - 0.116)	0.115 (0.11 - 0.116)
Dry and Cold Location	0.126 (0.121 - 0.127)	0.116 (0.11 - 0.117)	0.116 (0.11 - 0.117)
Average Rainfall and Warm Location	0.121 (0.114 - 0.124)	0.113 (0.108 - 0.114)	0.113 (0.108 - 0.115)
Average Rainfall and Temperate Location	0.124 (0.114 - 0.125)	0.114 (0.108 - 0.116)	0.115 (0.109 - 0.116)
Average Rainfall and Cool Location	0.124 (0.117 - 0.126)	0.115 (0.109 - 0.116)	0.115 (0.11 - 0.116)
Wet and Warm Location	0.117 (0.104 - 0.121)	0.11 (0.103 - 0.113)	0.113 (0.107 - 0.115)
Wet and Temperate Location	0.119 (0.109 - 0.123)	0.112 (0.107 - 0.115)	0.115 (0.109 - 0.116)
Wet and Cool Location	0.121 (0.109 - 0.125)	0.114 (0.109 - 0.116)	0.115 (0.11 - 0.117)
Average of Central Values:	0.122	0.114	0.115
25th Percentile:	0.121	0.113	0.114
Maximum:	0.127	0.117	0.117
Summary:	0.122 (0.121 - 0.127)	0.114 (0.113 - 0.117)	0.115 (0.114 - 0.117)

One Application

Table A8-4: Maximum Penetration into Soil Column (inches)

Site	Clay	Loam	Sand
Dry and Warm Location	8 (4 - 8)	8 (4 - 8)	8 (4 - 12)
Dry and Temperate Location	8 (4 - 8)	8 (4 - 12)	8 (4 - 12)
Dry and Cold Location	4 (4 - 8)	8 (4 - 8)	8 (4 - 12)
Average Rainfall and Warm Location	8 (8 - 12)	8 (8 - 12)	12 (8 - 18)
Average Rainfall and Temperate Location	8 (8 - 12)	8 (8 - 12)	12 (8 - 18)
Average Rainfall and Cool Location	8 (8 - 12)	8 (8 - 12)	8 (8 - 18)
Wet and Warm Location	8 (8 - 12)	8 (8 - 18)	12 (8 - 30)
Wet and Temperate Location	8 (8 - 18)	8 (8 - 18)	12 (8 - 30)
Wet and Cool Location	8 (8 - 12)	8 (8 - 18)	12 (8 - 30)
Average of Central Values:	7.56	8	10.2
25th Percentile:	8	8	8
Maximum:	18	18	30
Summary:	7.56 (8 - 18)	8 (8 - 18)	10.2 (8 - 30)

Appendix 8: GLEAMS-Driver, Single Application

One Application

Table A8-5: Stream, Maximum Peak Concentration in Surface Water (ug/L or ppb)

Site	Clay	Loam	Sand
Dry and Warm Location	1.76 (0 - 12.3)	0 (0 - 4.7)	0 (0 - 0)
Dry and Temperate Location	1.95 (0.04 - 21.6)	0.15 (0 - 10.6)	0 (0 - 0.6)
Dry and Cold Location	0.4 (0.06 - 6.4)	0 (0 - 0.4)	0 (0 - 0)
Average Rainfall and Warm Location	14.4 (3.6 - 67)	10.4 (1.9 - 41)	1.24 (0 - 18.1)
Average Rainfall and Temperate Location	10.4 (2.52 - 62)	6.3 (0.9 - 32)	0.25 (0 - 9.2)
Average Rainfall and Cool Location	5.1 (1.91 - 44)	2.31 (0.5 - 15.1)	0 (0 - 2.51)
Wet and Warm Location	30.3 (9.2 - 106)	26 (8.2 - 146)	8.4 (1.34 - 63)
Wet and Temperate Location	19.2 (6.7 - 64)	14.1 (4.8 - 65)	3.02 (0.4 - 19.7)
Wet and Cool Location	13.5 (4.6 - 54)	8.3 (2.6 - 31.2)	1 (0.12 - 9.1)
Average of Central Values:	10.8	7.51	1.55
25th Percentile:	1.95	0.15	0
Maximum:	106	146	63
Summary:	10.8 (1.95 - 106)	7.51 (0.15 - 146)	1.55 (0 - 63)

Appendix 8: GLEAMS-Driver, Single Application

One Application

Table A8-6: Stream, Annual Average Concentration in Surface Water (ug/L or ppb)

Site	Clay	Loam	Sand
Dry and Warm Location	0.016 (0 - 0.11)	0 (0 - 0.03)	0 (0 - 0)
Dry and Temperate Location	0.02 (0.0003 - 0.24)	0.0009 (0 - 0.06)	0 (0 - 0.004)
Dry and Cold Location	0.005 (0.0006 - 0.06)	0 (0 - 0.002)	0 (0 - 0)
Average Rainfall and Warm Location	0.3 (0.13 - 1.54)	0.16 (0.03 - 0.6)	0.011 (0 - 0.13)
Average Rainfall and Temperate Location	0.25 (0.1 - 1.4)	0.08 (0.017 - 0.4)	0.0018 (0 - 0.09)
Average Rainfall and Cool Location	0.15 (0.07 - 0.9)	0.03 (0.009 - 0.12)	0 (0 - 0.016)
Wet and Warm Location	0.9 (0.4 - 2.24)	0.5 (0.23 - 1.14)	0.09 (0.022 - 0.4)
Wet and Temperate Location	0.7 (0.4 - 1.95)	0.3 (0.18 - 0.9)	0.03 (0.006 - 0.19)
Wet and Cool Location	0.6 (0.27 - 1.87)	0.21 (0.09 - 0.6)	0.012 (0.0015 - 0.08)
Average of Central Values:	0.33	0.142	0.0161
25th Percentile:	0.02	0.0009	0
Maximum:	2.24	1.14	0.4
Summary:	0.33 (0.02 - 2.24)	0.142 (0.0009 - 1.14)	0.0161 (0 - 0.4)

Appendix 8: GLEAMS-Driver, Single Application

One Application

Table A8-7: Pond, Maximum Peak Concentration in Surface Water (ug/L or ppb)

Site	Clay	Loam	Sand
Dry and Warm Location	1.33 (0 - 9.7)	0 (0 - 2.63)	0 (0 - 0)
Dry and Temperate Location	1.6 (0.019 - 14.9)	0.06 (0 - 4.5)	0 (0 - 0.24)
Dry and Cold Location	0.4 (0.04 - 5.2)	0 (0 - 0.16)	0 (0 - 0)
Average Rainfall and Warm Location	42 (16.4 - 162)	17.6 (3.8 - 60)	1.29 (0 - 12.6)
Average Rainfall and Temperate Location	28.8 (10 - 172)	9.3 (1.92 - 48)	0.23 (0 - 13)
Average Rainfall and Cool Location	17.5 (7.9 - 96)	3.7 (1.12 - 16.6)	0 (0 - 3.3)
Wet and Warm Location	38 (19 - 94)	19.2 (10.9 - 41)	2.89 (0.6 - 16.2)
Wet and Temperate Location	18.4 (9.3 - 46)	7.5 (4.5 - 15.5)	0.7 (0.15 - 3.09)
Wet and Cool Location	20.1 (10.8 - 68)	8 (4.2 - 16.6)	0.5 (0.08 - 3.2)
Average of Central Values:	18.7	7.26	0.62
25th Percentile:	1.6	0.06	0
Maximum:	172	60	16.2
Summary:	18.7 (1.6 - 172)	7.26 (0.06 - 60)	0.62 (0 - 16.2)

One Application

Table A8-8: Pond, Annual Average Concentration in Surface Water (ug/L or ppb)

Site	Clay	Loam	Sand
Dry and Warm Location	0.4 (0 - 3.16)	0 (0 - 0.8)	0 (0 - 0)
Dry and Temperate Location	0.5 (0.008 - 6.8)	0.005 (0 - 1.59)	0 (0 - 0.04)
Dry and Cold Location	0.14 (0.012 - 1.66)	0 (0 - 0.05)	0 (0 - 0)
Average Rainfall and Warm Location	13.5 (4.2 - 59)	5.7 (1.32 - 22.5)	0.4 (0 - 6.6)
Average Rainfall and Temperate Location	9.4 (3.7 - 67)	3.2 (0.8 - 16)	0.07 (0 - 2.94)
Average Rainfall and Cool Location	6.7 (2.78 - 29.8)	1.32 (0.3 - 4.9)	0 (0 - 0.9)
Wet and Warm Location	11.6 (5.9 - 29.7)	6.4 (3.9 - 11.2)	0.9 (0.18 - 4.8)
Wet and Temperate Location	6.1 (3.3 - 14.9)	2.51 (1.5 - 4.8)	0.16 (0.022 - 0.9)
Wet and Cool Location	6.1 (3.13 - 22.6)	2.2 (1.19 - 4.7)	0.12 (0.015 - 0.7)
Average of Central Values:	6.05	2.37	0.183
25th Percentile:	0.5	0.005	0
Maximum:	67	22.5	6.6
Summary:	6.05 (0.5 - 67)	2.37 (0.005 - 22.5)	0.183 (0 - 6.6)

Appendix 9: Gleams-Driver Modeling, Two Applications

Two Applications with 6-Day Interval

Table A9-1: Effective Offsite Application Rate (lb/acre)

Site	Clay	Loam	Sand
Dry and Warm Location	0.00272 (0 - 0.0204)	0 (0 - 0.0051)	0 (0 - 0)
Dry and Temperate Location	0.0033 (0.000038 - 0.051)	0.000123 (0 - 0.011)	0 (0 - 0.00152)
Dry and Cold Location	0.00082 (0.000072 - 0.01)	0 (0 - 0.000313)	0 (0 - 0)
Average Rainfall and Warm Location	0.088 (0.034 - 0.306)	0.036 (0.0073 - 0.132)	0.00278 (0 - 0.037)
Average Rainfall and Temperate Location	0.058 (0.0207 - 0.307)	0.0192 (0.0038 - 0.097)	0.0004 (0 - 0.0287)
Average Rainfall and Cool Location	0.038 (0.0181 - 0.191)	0.0082 (0.00231 - 0.036)	0 (0 - 0.0065)
Wet and Warm Location	0.251 (0.121 - 0.62)	0.162 (0.074 - 0.39)	0.0255 (0.0061 - 0.134)
Wet and Temperate Location	0.219 (0.118 - 0.63)	0.113 (0.056 - 0.279)	0.0114 (0.00138 - 0.065)
Wet and Cool Location	0.206 (0.084 - 0.58)	0.074 (0.0303 - 0.206)	0.0046 (0.00064 - 0.0294)
Average of Central Values:	0.096	0.046	0.005
25th Percentile:	0.0033	1.23E-04	0
Maximum:	0.63	0.39	0.134
Summary:	0.096 (0.0033 - 0.63)	0.046 (1.23E-04 - 0.39)	0.005 (0 - 0.134)

Two Applications with 6-Day Interval

Table A9-2: Concentration in Top 12 Inches of Soil (ppm)

Site	Clay	Loam	Sand
Dry and Warm Location	0.74 (0.71 - 0.75)	0.68 (0.65 - 0.69)	0.68 (0.65 - 0.69)
Dry and Temperate Location	0.75 (0.72 - 0.81)	0.69 (0.66 - 0.75)	0.69 (0.66 - 0.75)
Dry and Cold Location	0.76 (0.72 - 0.82)	0.7 (0.66 - 0.76)	0.7 (0.66 - 0.76)
Average Rainfall and Warm Location	0.73 (0.68 - 0.78)	0.68 (0.65 - 0.74)	0.68 (0.65 - 0.74)
Average Rainfall and Temperate Location	0.74 (0.69 - 0.8)	0.69 (0.65 - 0.75)	0.69 (0.66 - 0.75)
Average Rainfall and Cool Location	0.74 (0.7 - 0.76)	0.69 (0.66 - 0.7)	0.69 (0.66 - 0.7)
Wet and Warm Location	0.7 (0.63 - 0.73)	0.66 (0.62 - 0.68)	0.68 (0.64 - 0.69)
Wet and Temperate Location	0.71 (0.65 - 0.79)	0.67 (0.64 - 0.74)	0.69 (0.66 - 0.75)
Wet and Cool Location	0.72 (0.62 - 0.75)	0.68 (0.65 - 0.69)	0.69 (0.66 - 0.7)
Average of Central Values:	0.73	0.68	0.69
25th Percentile:	0.72	0.68	0.68
Maximum:	0.82	0.76	0.76
Summary:	0.73 (0.72 - 0.82)	0.68 (0.68 - 0.76)	0.69 (0.68 - 0.76)

Two Applications with 6-Day Interval

Table A9-3: Concentration in Top 36 Inches of Soil (ppm)

Site	Clay	Loam	Sand
Dry and Warm Location	0.248 (0.237 - 0.25)	0.228 (0.217 - 0.23)	0.228 (0.218 - 0.23)
Dry and Temperate Location	0.25 (0.239 - 0.271)	0.23 (0.219 - 0.251)	0.23 (0.219 - 0.251)
Dry and Cold Location	0.252 (0.241 - 0.272)	0.232 (0.221 - 0.253)	0.232 (0.221 - 0.253)
Average Rainfall and Warm Location	0.243 (0.227 - 0.261)	0.225 (0.215 - 0.245)	0.227 (0.217 - 0.247)
Average Rainfall and Temperate Location	0.247 (0.228 - 0.267)	0.229 (0.216 - 0.249)	0.23 (0.219 - 0.25)
Average Rainfall and Cool Location	0.248 (0.233 - 0.252)	0.23 (0.219 - 0.233)	0.23 (0.22 - 0.233)
Wet and Warm Location	0.234 (0.208 - 0.243)	0.219 (0.205 - 0.226)	0.226 (0.214 - 0.229)
Wet and Temperate Location	0.238 (0.217 - 0.264)	0.225 (0.214 - 0.247)	0.229 (0.219 - 0.251)
Wet and Cool Location	0.24 (0.207 - 0.248)	0.227 (0.217 - 0.231)	0.231 (0.22 - 0.233)
Average of Central Values:	0.244	0.227	0.229
25th Percentile:	0.24	0.225	0.228
Maximum:	0.272	0.253	0.253
Summary:	0.244 (0.24 - 0.272)	0.227 (0.225 - 0.253)	0.229 (0.228 - 0.253)

Two Applications with 6-Day Interval

Table A9-4: Maximum Penetration into Soil Column (inches)

Site	Clay	Loam	Sand
Dry and Warm Location	8 (4 - 8)	8 (4 - 8)	8 (4 - 12)
Dry and Temperate Location	8 (4 - 12)	8 (4 - 12)	8 (8 - 12)
Dry and Cold Location	8 (4 - 8)	8 (4 - 12)	8 (8 - 12)
Average Rainfall and Warm Location	8 (8 - 12)	8 (8 - 18)	12 (8 - 24)
Average Rainfall and Temperate Location	8 (8 - 12)	8 (8 - 18)	12 (8 - 18)
Average Rainfall and Cool Location	8 (8 - 12)	8 (8 - 12)	12 (8 - 18)
Wet and Warm Location	8 (8 - 18)	12 (8 - 18)	12 (12 - 30)
Wet and Temperate Location	8 (8 - 18)	8 (8 - 24)	12 (12 - 36)
Wet and Cool Location	8 (8 - 18)	12 (8 - 18)	12 (8 - 30)
Average of Central Values:	8	8.89	10.7
25th Percentile:	8	8	8
Maximum:	18	24	36
Summary:	8 (8 - 18)	8.89 (8 - 24)	10.7 (8 - 36)

Two Applications with 6-Day Interval

Table A9-5: Stream, Maximum Peak Concentration in Surface Water (ug/L or ppb)

Site	Clay	Loam	Sand
Dry and Warm Location	3.5 (0 - 24.6)	0 (0 - 9.4)	0 (0 - 0)
Dry and Temperate Location	3.9 (0.09 - 43)	0.31 (0 - 21.2)	0 (0 - 1.13)
Dry and Cold Location	0.9 (0.12 - 12.9)	0 (0 - 0.8)	0 (0 - 0)
Average Rainfall and Warm Location	28.9 (7.2 - 134)	20.3 (3.8 - 83)	2.47 (0 - 36)
Average Rainfall and Temperate Location	20.5 (5 - 123)	12.1 (1.82 - 68)	0.5 (0 - 20.4)
Average Rainfall and Cool Location	10.1 (3.8 - 88)	4.4 (1.02 - 30.3)	0 (0 - 5)
Wet and Warm Location	61 (18.5 - 213)	52 (16.4 - 294)	16.7 (2.69 - 128)
Wet and Temperate Location	39 (14.1 - 129)	28.2 (9.5 - 130)	6.1 (0.8 - 40)
Wet and Cool Location	27 (9.1 - 109)	16.3 (5 - 73)	2.03 (0.22 - 18.8)
Average of Central Values:	21.6	14.8	3.09
25th Percentile:	3.9	0.31	0
Maximum:	213	294	128
Summary:	21.6 (3.9 - 213)	14.8 (0.31 - 294)	3.09 (0 - 128)

Two Applications with 6-Day Interval

Table A9-6: Stream, Annual Average Concentration in Surface Water (ug/L or ppb)

Site	Clay	Loam	Sand
Dry and Warm Location	0.03 (0 - 0.21)	0 (0 - 0.06)	0 (0 - 0)
Dry and Temperate Location	0.04 (0.0006 - 0.5)	0.0018 (0 - 0.12)	0 (0 - 0.008)
Dry and Cold Location	0.01 (0.0011 - 0.13)	0 (0 - 0.004)	0 (0 - 0)
Average Rainfall and Warm Location	0.7 (0.26 - 3.09)	0.31 (0.07 - 1.14)	0.022 (0 - 0.27)
Average Rainfall and Temperate Location	0.5 (0.19 - 2.72)	0.17 (0.03 - 0.9)	0.003 (0 - 0.2)
Average Rainfall and Cool Location	0.29 (0.14 - 1.66)	0.06 (0.019 - 0.25)	0 (0 - 0.03)
Wet and Warm Location	1.71 (0.8 - 4.5)	1 (0.5 - 2.28)	0.18 (0.05 - 0.9)
Wet and Temperate Location	1.39 (0.8 - 3.9)	0.7 (0.4 - 1.78)	0.06 (0.012 - 0.4)
Wet and Cool Location	1.18 (0.5 - 3.7)	0.4 (0.17 - 1.14)	0.024 (0.0029 - 0.17)
Average of Central Values:	0.65	0.294	0.032
25th Percentile:	0.04	0.0018	0
Maximum:	4.5	2.28	0.9
Summary:	0.65 (0.04 - 4.5)	0.294 (0.0018 - 2.28)	0.032 (0 - 0.9)

Two Applications with 6-Day Interval

Table A9-7: Pond, Maximum Peak Concentration in Surface Water (ug/L or ppb)

Site	Clay	Loam	Sand
Dry and Warm Location	2.76 (0 - 19.5)	0 (0 - 5.3)	0 (0 - 0)
Dry and Temperate Location	3.2 (0.04 - 29.8)	0.12 (0 - 8.9)	0 (0 - 0.5)
Dry and Cold Location	0.8 (0.07 - 10.4)	0 (0 - 0.3)	0 (0 - 0)
Average Rainfall and Warm Location	81 (33 - 320)	35 (7.4 - 120)	2.57 (0 - 25.2)
Average Rainfall and Temperate Location	58 (20.1 - 340)	18.2 (3.8 - 97)	0.4 (0 - 26)
Average Rainfall and Cool Location	34 (15.7 - 192)	7.5 (2.23 - 33)	0 (0 - 6.6)
Wet and Warm Location	76 (36 - 184)	38 (21.6 - 83)	5.8 (1.24 - 33)
Wet and Temperate Location	38 (18.7 - 92)	15 (9 - 31.1)	1.43 (0.3 - 6.2)
Wet and Cool Location	40 (21.3 - 139)	16 (8.4 - 34)	1.02 (0.12 - 5.6)
Average of Central Values:	37.1	14.4	1.25
25th Percentile:	3.2	0.12	0
Maximum:	340	120	33
Summary:	37.1 (3.2 - 340)	14.4 (0.12 - 120)	1.25 (0 - 33)

Two Applications with 6-Day Interval

Table A9-8: Pond, Annual Average Concentration in Surface Water (ug/L or ppb)

Site	Clay	Loam	Sand
Dry and Warm Location	0.7 (0 - 6.3)	0 (0 - 1.56)	0 (0 - 0)
Dry and Temperate Location	1 (0.016 - 13.6)	0.01 (0 - 3.2)	0 (0 - 0.09)
Dry and Cold Location	0.28 (0.024 - 3.3)	0 (0 - 0.11)	0 (0 - 0)
Average Rainfall and Warm Location	27.3 (8.4 - 118)	11.3 (2.64 - 45)	0.8 (0 - 13.3)
Average Rainfall and Temperate Location	19.2 (7.4 - 134)	6.2 (1.66 - 32)	0.13 (0 - 5.9)
Average Rainfall and Cool Location	12.9 (5.5 - 59)	2.6 (0.7 - 9.7)	0 (0 - 1.84)
Wet and Warm Location	23.2 (11.8 - 59)	12.9 (7.4 - 22.6)	1.82 (0.4 - 9.6)
Wet and Temperate Location	12.2 (6.5 - 29.8)	5 (2.99 - 9.7)	0.3 (0.04 - 1.74)
Wet and Cool Location	12 (6.1 - 42)	4.4 (2.35 - 9.3)	0.25 (0.027 - 1.4)
Average of Central Values:	12.1	4.71	0.37
25th Percentile:	1	0.01	0
Maximum:	134	45	13.3
Summary:	12.1 (1 - 134)	4.71 (0.01 - 45)	0.37 (0 - 13.3)

Appendix 10: EPA Surface Water Models

Input[1]	Central	Lower Bound Run	Upper Bound Run
Application rate (lb a.i./acre)	1	1	1
Proportion of Area Treated	1	1	1
K_{oc}	4237	134583	831
Soil aerobic half-time	0	0	0
Wetted in	No	No	No
Drift/Application Efficiency	0%/100%	0%/100%	0%/100%
Incorporation depth (cm)	0	0	0
Water Solubility (mg/L)	89.4	89.4	89.4
Aerobic aquatic half-life (days)[4]	0	0	0
Proportion of Area Treated	1	1	1

FIRST Output (µg/L)	Peak	Annual Average
Single Application		
Central Estimate	23.0	6.5
Lower Bound	17.0	0.52
Upper Bound	43.4	11.0
Two Applications, 6 Day Interval		
Central Estimate	46.0	13.1
Lower Bound	33.9	1.0
Upper Bound	86.8	22.0

PRZM-GW Output (µg/L)	Peak
Single Application	
Central Estimate	0.03
Lower Bound	0.006
Upper Bound	0.7
Two Applications, 6 day interval	
Central Estimate	0.06
Lower Bound	0.012
Upper Bound	1.4

[1] See inputs for GLEAMS-Driver in Table XX of risk assessment. The only exceptions are half-lives for soil and water aerobic half-lives set to zero to designate no degradation. This convention is not available in GLEAMS and half-times of 7,300 days are used for the GLEAMS modeling.

FIRST Output Files

Single Application

CENTRAL ESTIMATE (Central Estimate of Koc)

RUN No. 1 FOR Spinosad ON None * INPUT VALUES *

RATE (#/AC) ONE(MULT)	No.APPS & INTERVAL	SOIL Koc	SOLUBIL (PPM)	APPL TYPE (%DRIFT)	%CROPPED AREA	INCORP (IN)
1.000(1.000)	1 1	4237.0	89.4	GRANUL(0.0)	100.0	0.0

FIELD AND RESERVOIR HALFLIFE VALUES (DAYS)

METABOLIC (FIELD)	DAYS UNTIL RAIN/RUNOFF	HYDROLYSIS (RESERVOIR)	PHOTOLYSIS (RES.-EFF)	METABOLIC (RESER.)	COMBINED (RESER.)
0.00	2	N/A	0.00-	0.00	0.00	0.00

UNTREATED WATER CONC (MICROGRAMS/LITER (PPB)) Ver 1.1.1 MAR 26, 2008

PEAK DAY (ACUTE) CONCENTRATION	ANNUAL AVERAGE (CHRONIC) CONCENTRATION
22.978	6.539

* *

LOWER BOUND (Upper Bound of Koc)

RUN No. 1 FOR Spinosal ON None * INPUT VALUES *

RATE (#/AC) ONE(MULT)	No.APPS & INTERVAL	SOIL Koc	SOLUBIL (PPM)	APPL TYPE (%DRIFT)	%CROPPED AREA	INCORP (IN)
1.000(1.000)	1 1	134583.0	89.4	GRANUL(0.0)	100.0	0.0

FIELD AND RESERVOIR HALFLIFE VALUES (DAYS)

METABOLIC (FIELD)	DAYS UNTIL RAIN/RUNOFF	HYDROLYSIS (RESERVOIR)	PHOTOLYSIS (RES.-EFF)	METABOLIC (RESER.)	COMBINED (RESER.)
0.00	2	N/A	0.00-	0.00	0.00	0.00

UNTREATED WATER CONC (MICROGRAMS/LITER (PPB)) Ver 1.1.1 MAR 26, 2008

PEAK DAY (ACUTE) CONCENTRATION	ANNUAL AVERAGE (CHRONIC) CONCENTRATION
16.963	0.523

* *

UPPER BOUND (Lower Bound of Koc)

RUN No. 1 FOR Spinosad ON None * INPUT VALUES *

RATE (#/AC) ONE(MULT)	No.APPS & INTERVAL	SOIL Koc	SOLUBIL (PPM)	APPL TYPE (%DRIFT)	%CROPPED AREA	INCORP (IN)
1.000(1.000)	1 1	831.0	89.4	GRANUL(0.0)	100.0	0.0

FIELD AND RESERVOIR HALFLIFE VALUES (DAYS)

METABOLIC (FIELD)	DAYS UNTIL RAIN/RUNOFF	HYDROLYSIS (RESERVOIR)	PHOTOLYSIS (RES.-EFF)	METABOLIC (RESER.)	COMBINED (RESER.)
0.00	2	N/A	0.00-	0.00	0.00	0.00

UNTREATED WATER CONC (MICROGRAMS/LITER (PPB)) Ver 1.1.1 MAR 26, 2008

PEAK DAY (ACUTE) CONCENTRATION	ANNUAL AVERAGE (CHRONIC) CONCENTRATION
43.404	11.001

* *

FIRST Output Files

Two Applications, 6-Day Interval

CENTRAL ESTIMATE (Central Estimate of Koc)

RUN No. 1 FOR Spinosad ON None * INPUT VALUES *

RATE (#/AC) ONE(MULT)	No.APPS & INTERVAL	SOIL Koc	SOLUBIL (PPM)	APPL TYPE (%DRIFT)	%CROPPED AREA	INCORP (IN)
1.000(2.000)	2 6	4237.0	89.4	GRANUL(0.0)	100.0	0.0

FIELD AND RESERVOIR HALFLIFE VALUES (DAYS)

METABOLIC (FIELD)	DAYS UNTIL RAIN/RUNOFF	HYDROLYSIS (RESERVOIR)	PHOTOLYSIS (RES.-EFF)	METABOLIC (RESER.)	COMBINED (RESER.)
0.00	2	N/A	0.00-	0.00	0.00	0.00

UNTREATED WATER CONC (MICROGRAMS/LITER (PPB)) Ver 1.1.1 MAR 26, 2008

PEAK DAY (ACUTE) CONCENTRATION	ANNUAL AVERAGE (CHRONIC) CONCENTRATION
45.955	13.078

* *

LOWER BOUND (Upper Bound of Koc)

RUN No. 1 FOR Spinosad ON None * INPUT VALUES *

RATE (#/AC) ONE(MULT)	No.APPS & INTERVAL	SOIL Koc	SOLUBIL (PPM)	APPL TYPE (%DRIFT)	%CROPPED AREA	INCORP (IN)
1.000(2.000)	2 6	134583.0	89.4	GRANUL(0.0)	100.0	1.0

FIELD AND RESERVOIR HALFLIFE VALUES (DAYS)

METABOLIC (FIELD)	DAYS UNTIL RAIN/RUNOFF	HYDROLYSIS (RESERVOIR)	PHOTOLYSIS (RES.-EFF)	METABOLIC (RESER.)	COMBINED (RESER.)
0.00	2	N/A	0.00-	0.00	0.00	0.00

UNTREATED WATER CONC (MICROGRAMS/LITER (PPB)) Ver 1.1.1 MAR 26, 2008

PEAK DAY (ACUTE) CONCENTRATION	ANNUAL AVERAGE (CHRONIC) CONCENTRATION
33.926	1.045

* *

UPPER BOUND (Lower Bound of Koc)

RUN No. 1 FOR Spinosad ON None * INPUT VALUES *

RATE (#/AC) ONE(MULT)	No.APPS & INTERVAL	SOIL Koc	SOLUBIL (PPM)	APPL TYPE (%DRIFT)	%CROPPED AREA	INCORP (IN)
1.000(2.000)	2 6	831.0	89.4	GRANUL(0.0)	100.0	0.0

FIELD AND RESERVOIR HALFLIFE VALUES (DAYS)

METABOLIC (FIELD)	DAYS UNTIL RAIN/RUNOFF	HYDROLYSIS (RESERVOIR)	PHOTOLYSIS (RES.-EFF)	METABOLIC (RESER.)	COMBINED (RESER.)
0.00	2	N/A	0.00-	0.00	0.00	0.00

UNTREATED WATER CONC (MICROGRAMS/LITER (PPB)) Ver 1.1.1 MAR 26, 2008

PEAK DAY (ACUTE) CONCENTRATION	ANNUAL AVERAGE (CHRONIC) CONCENTRATION
86.808	22.002

* *

SciGrow version 2.3 Output files

Single Application

CENTRAL ESTIMATE (Central Estimate of Koc)

```
 SciGrow version 2.3
 chemical:Spinosad
 time is  4/ 4/2016  19:28:43
 ---------------------------------------------------------------------
  Application      Number of       Total Use    Koc      Soil Aerobic
  rate (lb/acre)  applications   (lb/acre/yr)  (ml/g)   metabolism (days)
 ---------------------------------------------------------------------
      1.000           1.0           1.000      4.24E+03     7300.0
 ---------------------------------------------------------------------
 groundwater screening cond (ppb) =   3.02E-02
 *********************************************************************

* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

LOWER BOUND (Upper Bound of Koc)
SciGrow version 2.3
 chemical:Spinosad
 time is  4/ 4/2016  19:29:30
 ---------------------------------------------------------------------
  Application      Number of       Total Use    Koc      Soil Aerobic
  rate (lb/acre)  applications   (lb/acre/yr)  (ml/g)   metabolism (days)
 ---------------------------------------------------------------------
      1.000           1.0           1.000      1.35E+05     7300.0
 ---------------------------------------------------------------------
 groundwater screening cond (ppb) =   6.00E-03*
 *Estimated concentrations of chemicals with Koc values greater than 9995 ml/g
 are beyond the scope of the regression data used in SCI-GROW development.
 If there are concerns for such chemicals, a higher tier groundwater exposure
 assessment should be considered, regardless of the concentration returned
 by SCI-GROW.
 *********************************************************************

* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

UPPER BOUND (Lower Bound of Koc)
SciGrow version 2.3
 chemical:Spinosad
 time is  4/ 4/2016  19:30: 0
 ---------------------------------------------------------------------
  Application      Number of       Total Use    Koc      Soil Aerobic
  rate (lb/acre)  applications   (lb/acre/yr)  (ml/g)   metabolism (days)
 ---------------------------------------------------------------------
      1.000           1.0           1.000      8.31E+02     7300.0
 ---------------------------------------------------------------------
 groundwater screening cond (ppb) =   7.03E-01
 *********************************************************************


* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
```

SciGrow version 2.3 Output files

Two Applications, 6 day interval

CENTRAL ESTIMATE (Central Estimate of Koc)

```
SciGrow version 2.3
 chemical:Spinosad
 time is  4/ 4/2016  19:34:24
 -------------------------------------------------------------------------
  Application      Number of       Total Use    Koc      Soil Aerobic
  rate (lb/acre)  applications   (lb/acre/yr)  (ml/g)   metabolism (days)
 -------------------------------------------------------------------------
      1.000           2.0           2.000      4.24E+03     7300.0
 -------------------------------------------------------------------------
 groundwater screening cond (ppb) =   6.05E-02
 *************************************************************************

* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
```

LOWER BOUND (Upper Bound of Koc)

```
SciGrow version 2.3
 chemical:Spinosad
 time is  4/ 4/2016  19:35:52
 -------------------------------------------------------------------------
  Application      Number of       Total Use    Koc      Soil Aerobic
  rate (lb/acre)  applications   (lb/acre/yr)  (ml/g)   metabolism (days)
 -------------------------------------------------------------------------
      1.000           2.0           2.000      1.35E+05     7300.0
 -------------------------------------------------------------------------
 groundwater screening cond (ppb) =   1.20E-02*
 *Estimated concentrations of chemicals with Koc values greater than 9995 ml/g
 are beyond the scope of the regression data used in SCI-GROW development.
 If there are concerns for such chemicals, a higher tier groundwater exposure
 assessment should be considered, regardless of the concentration returned
 by SCI-GROW.
 *************************************************************************

* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
```

UPPER BOUND (Lower Bound of Koc)

```
SciGrow version 2.3
 chemical:Spinosad
 time is  4/ 4/2016  19:36:28
 -------------------------------------------------------------------------
  Application      Number of       Total Use    Koc      Soil Aerobic
  rate (lb/acre)  applications   (lb/acre/yr)  (ml/g)   metabolism (days)
 -------------------------------------------------------------------------
      1.000           2.0           2.000      8.31E+02     7300.0
 -------------------------------------------------------------------------
 groundwater screening cond (ppb) =   1.41E+00
 *************************************************************************

* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
```